EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L2	1	1 same multip$	US-PGPUB; USPAT	ADJ	ON	2006/02/03 14:43
L3	45	nitrous acid same extract$ same antigen	US-PGPUB; USPAT	ADJ	ON	2006/02/03 15:07
L4	0	l3 and virus]	US-PGPUB; USPAT	ADJ	ON	2006/02/03 15:07
L5	24	l3 and virus	US-PGPUB; USPAT	ADJ	ON	2006/02/03 15:08
L6	0	nitrous acid extraction same virus	US-PGPUB; USPAT	ADJ	ON	2006/02/03 15:08

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 NEWS  9  JAN 13  IPC 8 searching in IFIPAT, IFIUDB, and IFICDB
 NEWS 10  JAN 13  New IPC 8 SEARCH, DISPLAY, and SELECT enhancements added to
                  INPADOC
 NEWS 11  JAN 17  Pre-1988 INPI data added to MARPAT
 NEWS 12  JAN 17  IPC 8 in the WPI family of databases including WPIFV
 NEWS 13  JAN 30  Saved answer limit increased
 NEWS 14  JAN 31  Monthly current-awareness alert (SDI) frequency
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=> index biosci
FILE 'DRUGMONOG' ACCESS NOT AUTHORIZED
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                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE,
       AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS,
       CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB,
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70 FILES IN THE FILE LIST IN STNINDEX

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L3   ANSWER 1 OF 17  IFIPAT  COPYRIGHT 2006 IFI on STN
AN    11039008  IFIPAT;IFIUDB;IFICDB
TI    SEMISYNTHETIC PROTEIN-BASED SITE-DIRECTED PROBES FOR IDENTIFICATION AND
      INHIBITION OF ACTIVE SITES, AND METHODS THEREFOR
INF   Borodovsky; Anna, Somerville, MA, US
      Galardy; Paul, North Andover, MA, US
      Gan-Erdene; Tudevin, Atlanta, GA, US
      Hemelaar; Joris, Boston, MA, US
      Kessler; Benedikt, Cambridge, MA, US
      Kolli; Nagamalleswari, Doraville, GA, US
      Ovaa; Huib, Boston, MA, US
      Ploegh; Hidde L., Brookline, MA, US
      Wilkinson; Keith D., Lilburn, GA, US
IN    Borodovsky Anna; Galardy Paul; Gan-Erdene Tudevin; Hemelaar Joris;
      Kessler Benedikt; Kolli Nagamalleswari; Ovaa Huib; Ploegh Hidde L;
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Wilkinson Keith D
PAF Unassigned
PA Unassigned Or Assigned To Individual (68000)
AG MINTZ, LEVIN, COHN, FERRIS, GLOVSKY;AND POPEO, P.C., ONE FINANCIAL CENTER, BOSTON, MA, 02111, US
PI US 2005277762 A1 20051215
AI US 2003-423421 20030425
PRAI US 2002-375586P 20020425 (Provisional)
FI US 2005277762 20051215
DT Utility; Patent Application - First Publication
FS CHEMICAL
APPLICATION
GOVI This invention was made in part with government support under grant numbers GM062502, GM30308, and GM66355 awarded by the National Institutes of Health. The Ad government has certain rights in the invention.
PARN This application claims the benefit of provisional application Ser. No. 60/375,586 filed Apr. 25, 2002, and which is incorporated herein by reference.
CLMN 30
GI 14 Figure(s).

FIG. 1A is sketch of chemical reactions for synthesis of UbVS Cterminal modification of ubiquitin, compound 1, to generate ubiquitin vinyl sulfone, compound 6. An amount of 25 mg of compound 1 was converted to ubiquitin-75 ethyl ester, compound 2, by treatment with 2.5 mg trypsin in presence of 1.6 M glycine ethyl ester (GlyOEt) and 20% PEG 20,000. Compound 2 was treated with hydrazine monohydrate and HCl to generate ubiquitin-75 hydrazine, compound 3, which was dialyzed against water and converted to ubiquitin-75 azide, compound 4, by treatment with 0.5 M ***nitrous*** ***acid*** for 1 min at-5 degrees C. Compound 4 was immediately reacted with the TFA salt of glycine vinyl sulfone, compound 5, in presence of TEA, generating compound 6. FIG. 1B is a readout of purified UbVS being resolved on an analytical C4 column using a 0.1% formic acid/ acetonitrile buffer system. Eluate was analyzed by on-line ESMS. The indicated multicharged species correspond to a molecular weight of 8624.9 Da, in agreement with the predicted MW of UbVS (8625 Da). FIG. 1C is a photograph of an SDS-PAGE characterization of a reaction in which recombinant purified UCH-L3 was incubated with a substoichiometric amount of (125I)UbVS in PBS for 45 min at 37 degrees C., with or without pretreatment with 2 mM N-ethylmaleimide. Protein conjugates were resolved by 12.5% SDS-PAGE under reducing conditions, and visualized by silver stain (left panel) and autoradiography (right panel).

FIG. 2A is a photograph of SDS-PAGE characterization of (125I)UbVS specifically labeling a subset of yeast DUBs from 100 mu g of post-nuclear lysates from DUB deletion strains that were incubated with 1x106 cpm of (125I)-UbVS for 45 min at 37 degrees C. Reactions were quenched with sample buffer, resolved by 10% SDS-PAGE and analyzed by autoradiography. Identity of bands was assigned based on the absence of a band corresponding to the molecular weight of a DUB deleted in that strain. FIG. 2B is a photograph of SDS-PAGE characterization showing lysates from wild type yeast that were pre-incubated with increasing concentrations of Ubal competitor for 30 min at room temperature, prior to addition of (125I)-UbVS and SDS-PAGE as described in FIG. 2A.

FIG. 3A is a photograph of SDS-PAGE characterization of (125I)UbVS labeling mammalian DUBs from single cell suspensions prepared from tissues of a male B6 mouse: muscle (MU), brain (BR), kidney (KI), thymus (TH), and spleen (SP). Lysate (50 mu g) was treated with 1x106 cpm (125I)-UbVS as described in FIG. 2A, and resolved by 10% SDS-PAGE. FIG. 3B is a photograph of SDS-PAGE characterization of 20 mu g of post-nuclear lysates from NIH 3T3 cells that were pre-treated with increasing concentrations of Ubal as competitor for 30 min at 37 degrees C. , followed by labeling with (125I)-UbVS. FIG. 3C is a photograph of SDS-PAGE characterization of 50 mu g of lysates from EL-4 cells that were treated with 2 mu M UbVS at 37 degrees C. for 1 hr, and resolved by 10% SDS-PAGE and immunoblotted with the r201 rabbit anti-serum against USP7.

FIG. 4A is a photograph of SDS-PAGE characterization of USP14 associating with the 26S proteasome, using EL-4 cell lysates fractionated on a Superose 6 FPLC column to isolate high molecular weight complexes, as described in Materials and methods. Fractions were labeled with 0.5x106 cpm of(125I)-UbVS and resolved by SDS-PAGE. FIG. 4B is a photograph of SDS-PAGE characterization of 80 mu g of EL4 cell lysates treated with 2x106 cpm (125I)-UbVS and immunoprecipitated with anti-20S proteasome anti-serum using proteasome IP buffer or denatured with 1% SDS and

immunoprecipitated with anti-USP14 antiserum HM433 using NET buffer. FIG. 4C is a photograph of SDS-PAGE characterization of EL4 lysates fractionated on a Superose 6 column as described in FIG. 4A. A volume of 1 ml of each fraction was incubated with (125I)-UbVS and immunoprecipitated for the proteasome as in B (top panel). A volume of 50 mu l of each fraction was analyzed for presence of proteasome subunits by immunoblot with indicated antibodies against components of the 20S and the 19S (lower panels).

FIG. 5A is a photograph of SDS-PAGE characterization of 125I)UbVS labeling of proteasome-associated USP14, which is shown to be increased upon proteasome inhibition, using 5x106 EL4 cells treated with 50 mu M NLVS for indicated times. Lysates were normalized for total protein and incubated with (125I)-UbVS or (125I)-NLVS for 1 hr. Proteasomes were immunoprecipitated as described in FIG. 4B. FIG. 5B is a bar graph that shows intensities of USP14 bands, quantified by densitometry, in untreated (black) and NLVS-treated (gray) cells. FIG. 5C is a photograph of SDS-PAGE characterization of EL4 cells that were incubated with 50 mu M NLVS, or ZL3VS, or 4 mu M epoxomicin for the indicated times. Proteasomes were immunoprecipitated as described in FIG. 4B.

FIG. 6A is a photograph of SDS-PAGE characterization showing that activity of USP14 is increased in response to proteasome inhibition, with subcellular fractions from EL-4 cells previously treated with 50 mu M NLVS for 5 hours, incubated with (125I)-UbVS, resolved by 10% SDS-PAGE, and visualized by autoradiography. The term 1hS means 1 hour supernatant, 5 hS means 5 hour supernatant, 5 hP means 5 hour pellet. FIG. 6B is a photograph of SDS-PAGE characterization of parallel samples resolved by 10% SDS-PAGE and inmnunoblotted with anti-USP14 and anti-Mss1 anti-sera. FIG. 6C is a bar graph that shows subcellular fractions prepared from EL4 cell ***extracts*** that were incubated with 50 mu M NLVS for 3 hours, resolved by 10% SDSPAGE, and visualized by autoradiography (upper panel), and by immunoblot using anti-USP14 (lower panel). Intensities of USP14 bands obtained from four independent experiments were quantified by densitometry and normalized to the amount of USP14 labeled by 125I)-UbVS (upper panel) and USP14 protein (lower panel) observed in 1 hour supernatant fractions.

FIG. 7 is a flow chart showing bacterial production of a fusion protein from an intein vector encoding a fusion protein having, from the direction of the amino terminus to the caarboxy terminus, an epitope tag such as hemagglutinin (HA), a peptide which is ubiquitin or a ubiquitin-like protein lacking the Cterminal amino acid, an intein, and a peptide capable of binding to an affinity material such as a chitin binding domain. Expression of the fusion protein in a microbial cell is regulated by the operon repressor LacZ, e.g., so that expression is induced by addition of IPTG. The protein is purified by a single step of affinity chromatography or batch purification on the basis of affinity to chitin immobilized on the column or on a suspension of beads. The protein is cleaved by treatment with the reagent mercapto ethane sulfonic acid sodium salt (MESNA), to release the ubiquitin or ubiquitin-like peptide portion as a thioester rather than having a C-terminal amino acid; the intein-containing portion is retained on the column or bead. As a result of an intramolecular rearrangement, the peptide at the carboxy terminus has an amine linked to a chemical group, R.

FIG. 8A is a drawing that shows the "new route" for synthesis of potential inhibitors, which comprises introduction of "warheads" following a series of chemical steps after cleavage with MESNA. Use of 1-amino-2,2-dimethoxyalkane followed by acid catalyzed hydrolysis of the resulting acetal leads to introduction of an aldehyde, which is a reversible inhibitor of a variety of ubiquitin-related enzymes. A further Wittig reaction with stabilized ylides of the obtained aldehydes in aqueous solution leads directly to peptide-michael acceptors having an electron withdrawing group (EWG), which can be an active irreversible Michael-acceptor inhibitor. FIG. 8B is a drawing that shows the Wittig reaction performed on an intact protein. The Wittig reaction allows for convenient and rapid introduction of variation in shape, electrophilicity and position of the electrophilic trap.

FIG. 9 is a drawing that shows the chemical structures of a series of inhibitors at the carboxy terminus of ubiquitin-gly75, synthesized by the initial route (left) involving direct chemical ligation of an electrophile at the thioester and by the new route (right) involving a wittig reaction on the corresponding aldehyde.

FIG. 10A is a drawing showing semi-synthetic construction of the HAUb vectors, which are constructs encoding HA-Ub peptide fusions with

C-terminal modifications, the drawing showing a choice of several different reactive groups. FIG. 10B shows the chemical structures of a series of reactive groups. FIG. 10C is a photograph of an SDS-PAGE analysis of an anti-hemagglutin (anti-HA) blot antibody blot (Western) that shows protein bands fractionated on the basis of molecular weight which are labeled as shown in FIG. 4 by a series of vectors derived from vector HAUb. A sample of 20 mu l of EL4 cell lysate was incubated with 0.5 mu M of each of vectors HAUb (control without a reactive group), HAUBVS, HAUbVMe, HAUbVSPh, and HAUbBr1, HAUbBr2, or 1 mu M of each of HAUbCN, HAUbC1, and HAUbBr3. Proteins labeled with the vectors were resolved by 8% reducing SDS-PAGE and immunoblotted with anti-HA antibody to reveal the bands shown in each lane. Data show that different HAUb-derived active sitedirected probes show distinct labeling profiles. FIG. 11 is a photograph of an electrophoretogram of proteins bound by different reactive site inhibitors using the HAUb vectors indicated in each lane. The proteins were immunoisolated using antibody specific for the HA epitope tag, were fractionated by electrophoresis performed under nondenaturing conditions, and were visualized with silver stain so that all proteins present are shown. Open circles indicate 19S cap bound USPs and open circles the 19S cap subunits. FIG. 12 is a family tree drawing of the relationships of sequences of the UBP family of enzymes. The catalytic domains of the UBPs annotated in SwissProt or GenBank databases or sequences herein were assigned based on ProSite parameters. Catalytic domains werw aligned using MegAlign program of the DNAStar software packing (using a Clustal V algorithm PAM250 Matrix); all catalytic residue signatures were well aligned, except CYLD1, for which a recognizable His box could not be found. A neighbor-joining phylogenetic tree was generated based on alignment. Shaded boxes indicate enzymes targeted by HAUb probes herein (HAUbVS, HAUbVME and HAUbBr2) that were identified by mass spectroscopy. FIG. 13 is a set of photographs of gel electrophoretograms showing the Ub1-Vs probes label distinct sets of proteins in EL4 lysates. FIG. 13A shows use of 125(I)-Ub-VS. FIG. 13B shows use of 125(I)-Nedd8-VS. FIG. 13C shows use of 125(I)-UCRP-VS. FIG. 13D shows use of 125(I)-SUMO-1-VS. Vinyl sulfone derivatives of each Ubl (ubiquitin-like proteins) were radiolabeled with Na125I and were incubated with EL-4 cell lysates. In each sample, 5x105 cpm of 125(I)-labeled probe and 40 mu g of EL-4 lysate were used. The left-most lane in each Fig. shows electrophoresis with no lysate added (probe alone control); remaining lanes show contents of EL-4 sample pretreatment as indicated on the top: EL-4 alone (no pretreatment), or pretreatment with 1 mM PMSF, 10 mM NEM, or 20 mM NEM, respectively, prior to addition of probe. Data show that the different Ubl probes have different profiles of protein interactions in the same cell type. FIG. 14 is a photograph of gel electrophoretogram of sets of different lymphocytic cell lines (LCLs) probed with HAUb probe HAUbVME. The lanes labeled LCLs are Epstein-Barr ***virus*** infected B-cell lines. Labeled bands were visualized with anti-HA immunoblot.! OF 17 IFIPAT COPYRIGHT 2006 IFI on STN

AB A C-terminally modified Ubiquitin (Ub) derivative) ubiquitin vinyl sulfone (UbVS), which is specific for deubiquitinating enzymes (DUBs), was synthesized as an active site directed probe that irreversibly modifies a subset of Ub C-terminal hydrolases (UCHs) and Ub specific processing proteases (UBPs), is provided. (125I)-UbVS modifies 6 of the 17 known and putative yeast deubiquitinating enzymes, namely Yuh1p, Ubp1p, Ubp2p, Ubp6p, Ubp12p and Ubp15p. In mammalian cells, a greater number of polypeptides is labeled, most of which are DUBs. An additional DUB that associates with the mammalian 26S proteasome, novel protein USP14, a mammalian homolog of yeast Ubp6p that is bound to the proteasome, is provided.

L3 ANSWER 2 OF 17 IFIPAT COPYRIGHT 2006 IFI on STN
AN 11021441 IFIPAT;IFIUDB;IFICDB
TI THERAPEUTIC AND COSMETIC USES OF HEPARANASES
INF Feinstein; Elena, Rehovot, IL
Ilan; Neta, Rehovot, IL
Pecker; Iris, Rishon LeZion, IL
Vlodavsky; Israel, Mevaseret Zion, IL
Yacoby-Zeevi; Oron, Moshav Bizaron, IL
IN Feinstein Elena (IL); Ilan Neta (IL); Pecker Iris (IL); Vlodavsky Israel (IL); Yacoby-Zeevi Oron (IL)
PAF Hadasit Medical Research Services and Development Ltd.

Insight Strategy & Marketing Ltd.
PA Hadasit Medical Res Services and Dev Ltd
Insight Strategy and Marketing Ltd
AG Martin MOYNIHAN;c/o ANTHONY CASTORINA, SUITE 207, 2001 JEFFERSON DAVIS HIGHWAY, ARLINGTON, VA, 22202, US
PI US 2005260187 A1 20051124
AI US 2005-106672 20050415
RLI US 1999-258892 19990301 CONTINUATION ABANDONED
US 2001-776874 20010206 CONTINUATION PENDING
US 2003-341582 20030114 CONTINUATION PENDING
WO 1998-US17954 19980831 CONTINUATION-IN-PART PENDING
US 2000-727479 20001204 CONTINUATION-IN-PART ABANDONED
WO 2001-IL830 20010905 CONTINUATION-IN-PART
US 2001-988113 20011119 CONTINUATION-IN-PART 6790658
PRAI US 2000-231551P 20000911 (Provisional)
US 2000-244593P 20001101 (Provisional)
FI US 2005260187 20051124
US 6790658
DT Utility; Patent Application - First Publication
FS CHEMICAL
APPLICATION
PARN This is a continuation of U.S. patent application Ser. No. 10/ 341,582, filed Jan. 14, 2003, which is a continuation-in-part of U.S. patent application Ser. No. 09/988,113, filed Nov. 19, 2001, now U.S. Pat. No. 6,790,658, which is a continuation of U. S. patent application Ser. No. 09/776,874, filed Feb. 6, 2001, which is a continuation of U.S. patent application Ser. No. 09/ 258,892, filed Mar. 1, 1999, now abandoned, which is a continuation-in-part of PCT/US98/17954, filed: Aug. 31, 1998, which claims priority from U.S. patent application Ser. No. 09/ 109,386, filed Jul. 2, 1998, now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 08/922, 170, filed Sep. 2, 1997, now U.S. Pat. No. 5,968,822. U.S. patent application Ser. No. 10/341,582 also was a continuation-in-part of PCT/IL01/00830, filed Sep. 5, 2001, whereby this application is also a continuation-in-part of PCT/ IL01/00830, filed Sep. 5, 2001, which claims the benefit of priority from U.S. patent application Ser. No. 09/727,479, filed Dec. 4, 2000, now abandoned, which claims the benefit of priority from U.S. Provisional Patent Application Nos. 60/231, 551, filed Sep. 11, 2000, and 60/244,593, filed Nov. 1, 2000.
CLMN 9
GI 36 Figure(s).

FIG. 1 presents nucleotide sequence and deduced amino acid sequence of hpa cDNA. A single nucleotide difference at position 799 (A to T) between the EST (Expressed Sequence Tag) and the PCR amplified cDNA (reverse transcribed RNA) and the resulting amino acid substitution (Tyr to Phe) are indicated above and below the substituted unit, respectively. Cysteine residues and the poly adenylation consensus sequence are underlined. The asterisk denotes the stop codon TGA.

FIG. 2 demonstrates degradation of soluble sulfate labeled HSPG substrate by lysates of High Five cells infected with pFhpa2 ***virus*** . Lysates of High Five cells that were infected with pFhpa2 ***virus*** (small-circle) or control pF2 ***virus*** , (.squ.) Were incubated (18 h, 37 degrees C.) with sulfate labeled ECMderived soluble HSPG (peak I). The incubation medium was then subjected to gel filtration on Sepharose 6B. Low molecular weight HS degradation fragments (peak II) were produced only during incubation with the pFhpa2 infected cells, but there was no degradation of the HSPG substrate (open-diamond) by lysates of pF2 infected cells.

FIGS. 3a-b demonstrate degradation of soluble sulfate labeled HSPG substrate by the culture medium of pFhpa2 and pFhpa4 infected cells. Culture media of High Five cells infected with pFhpa2 (3a) or pFhpa4 (3b) ***viruses*** (small-circle), or with control ***viruses*** (.squ.) were incubated (18 h, 37 degrees C.) with sulfate labeled ECM-derived soluble HSPG (Peak I, opendiamond). The incubation media were then subjected to gel filtration on Sepharose 6B. Low molecular weight HS degradation fragments (peak II) were produced only during incubation with the hpa gene containing ***viruses*** . There was no degradation of the HSPG substrate by the culture medium of cells infected with control ***viruses*** .

FIG. 4 presents size fractionation of heparanase activity expressed by pFhpa2 infected cells. Culture medium of pFhpa2 infected High Five &ells was applied onto a 50 kDa cut-off membrane. Heparanase activity

(conversion of the peak I substrate, (open-diamond) into peak II HS degradation fragments) was found in the high (>50 kDa) (small-circle), but not low (<50 kDa) (composite) molecular weight compartment.

FIGS. 5a-b demonstrate the effect of heparin on heparanase activity expressed by pFhpa2 and pFhpa4 infected High Five cells. Culture media of pFhpa2 (5a) and pFhpa4 (5b) infected High Five cells were incubated (18 h, 37 degrees C.) with sulfate labeled ECM-derived soluble HSPG (peak I, open-diamond) in the absence (small-circle) or presence (Delta) of 10 mu g/ml heparin. Production of low molecular weight HS degradation fragments was completely abolished in the presence of heparin, a potent inhibitor of heparanase activity (6, 7).

FIGS. 6a-b demonstrate degradation of sulfate labeled intact ECM by ***virus*** infected High Five and Sf21 cells. High Five (6a) and Sf21 (6b) cells were plated on sulfate labeled ECM and infected (48 h, 28 degrees C.) with pFhpa4 (small-circle) or control pF1 (.squ.) ***viruses*** . Control non-infected Sf2.1 cells (R) were plated on the labeled ECM as well. The pH of the cultured medium was adjusted to 6.0-6.2 followed by 24 h incubation at 37 degrees C. Sulfate labeled material released into the incubation medium was analyzed by gel filtration on Sepharose 6B. HS degradation fragments were produced only by cells infected with the hpa containing ***virus*** .

FIG. 7a-b demonstrate degradation of (sec) sulfate labeled intact ECM by ***virus*** infected cells. High Five (7a) and Sf21 (7b) cells were plated on sulfate labeled ECM and infected (48 h, 28 degrees C.) with pFhpa4 (small-circle) or control pF1 (.squ.) ***viruses*** . Control non-infected Sf21 cells (R) were plate on labeled ECM as well. The pH of the cultured medium was adjusted to 6.0-6.2, followed by 48 h incubation at 28 degrees C. Sulfate labeled degradation fragments released into the incubation medium was analyzed by gel filtration on. Sepharose 6B. HS degradation fragments were produced only by cells infected with the hpa containing ***virus*** .

FIGS. 8a-b demonstrate degradation of sulfate labeled intact ECM by the culture medium of pFhpa4 infected cells. Culture media of High Five (8a) and Sf21 (8b) cells that were infected with pFhpa4 (small-circle) or control pF1 (.squ.) ***viruses*** were incubated (48 h, 37 degrees C., pH 6.0) with intact sulfate labeled ECM. The ECM was also incubated with the culture medium of control non-infected Sf21 cells (R). Sulfate labeled material released into the reaction mixture was subjected to gel filtration analysis. Heparanase activity was detected only in the culture medium of pFhpa4 infected cells.

FIGS. 9a-b demonstrate the effect of heparin on heparanase activity in the culture medium of pFhpa4 infected cells. Sulfate labeled ECM was incubated (24 h, 37 degrees C., pH 6.0) with culture medium of pFhpa4 infected High Five (9a) and Sf21 (9b) cells in the absence (small-circle) or presence (V) of 10 mu g/mim heparin. Sulfate labeled material released into the incubation medium was subjected to gel filtration on-Sepharose 6B. Heparanase activity (production of peak II HS degradation fragments) was completely inhibited in the presence of heparin.

FIGS. 10a-b demonstrate purification of recombinant heparanase on heparin-Sepharose. Culture medium of Sf21 cells infected with pFhpa4 ***virus*** was subjected to heparin-Sepharose chromatography. Elution of fractions was performed with 0.35-2 M NaCl gradient (open-diamond). Heparanase activity in the eluted fractions is demonstrated in FIG. 10a (small-circle). Fractions 15-28 were subjected to 15% SDS-polyacrylamide gel electrophoresis followed by silver nitrate staining. A correlation is demonstrated between a major protein band (MW 63, 000) in fractions 19-24 and heparanase activity.

FIGS. 11a-b demonstrate purification of recombinant heparanase on a Superdex 75 gel filtration column. Active fractions eluted from heparin-Sepharose (FIG. 10a) were pooled, concentrated and applied onto Superdex 75 FPLC column. Fractions were collected and aliquots of each fraction were tested for heparanase activity (C,

FIG. 11a) and analyzed by SDS-polyacrylamide gel electrophoresis followed by silver nitrate staining (FIG. 11b). A correlation is seen between the appearance of a major protein band (MW 63, 000) in fractions 4-7 and heparanase activity.

FIGS. 12a-e demonstrate expression of the hpa gene by RT-PCR with total RNA from human embryonal tissues (12a), human extraembryonal tissues (12b) and cell lines from different origins (12c-e). RT-PCR products using hpa specific primers (I), primers for GAPDH housekeeping gene (II), and control reactions without reverse transcriptase demonstrating absence of genomic DNA or other contamination in RNA samples (III). M-DNA

molecular weight marker VI (Boehringer Mannheim). For 12a: lane 1 neutrophil cells (adult), lane 2 muscle, lane 3 thymus, lane 4 heart, lane 5 adrenal. For 12b: lane 1 kidney, lane 2 placenta. (8 weeks), lane 3 placenta (11 weeks), lanes 4-7 mole (complete hydatidiform mole), lane 8 cytotrophoblast cells (freshly isolated), lane 9 cytotrophoblast cells (1.5 h in vitro), lane 10 cytotrophoblast cells (6 h in vitro), lane 11 cytotrophoblast cells (18 h in vitro), lane 12 cytotrophoblast cells (48 h in vitro). For 12c. lane 1 JAR bladder cell line, lane 2 NCITT testicular tumor cell line, lane 3 SW-480 human hepatoma cell line, lane 4 HTR (cytotrophoblasts transformed by SV40), lane 5 HPTLP-I hepatocellular carcinoma cell line, lane 6 EJ-28 bladder carcinoma cell line. For 12d: lane 1 SK-hep-1 human hepatoma cell line, lane 2 DAMI human megakaryocytic cell line, lane 3 DAMI cell line+PMA, lane 4 CHRF cell line+PMA, lane 5 CHRF cell line. For 12e: lane 1 ABAE bovine aortic endothelial cells, lane 2-1063 human ovarian cell line, lane 0. 3 human breast carcinoma MDA435 cell line, lane 4 human breast carcinoma MDA231 cell line.

FIG. 13 presents a comparison between nucleotide sequences of the human hpa and a mouse EST cDNA fragment (SEQ ID NO:12) which is 80% homologous to the 3' end (starting at nucleotide 1066' of SEQ ID NO:9) of the human hpa the aligned termination codons are underlined.

FIG. 14 demonstrates the chromosomal localization of the hpa gene. PCR products of DNA derived from somatic cell hybrids and of genomic DNA of hamster, mouse and human of were separated on: 0.7% agarose gel following amplification with hpa specific primers. Lane 1 Lambda DNA digested with BstEII, lane 2 no DNA control, lanes 3-29, PCR amplification products. Lanes 3-5 human, mouse and hamster genomic DNA, respectively. Lanes 6-29, human monochromosomal somatic cell hybrids representing chromosomes 1-22 and X and Y, respectively. Lane 30 Lambda DNA digested with BstEII. An amplification product of approximately 2.8 Kb is observed only in lanes 5 and 9, representing human genomic DNA and DNA derived from cell hybrid carrying human chromosome 4, respectively. These results demonstrate that the hpa gene is localized in human chromosome 4.

FIG. 15 demonstrates the genomic exon-intron structure of the human hpa locus (top) and the relative positions of the lambda clones used as sequencing templates to sequence the locus (below). The vertical rectangles represent exons (E) and the horizontal lines therebetween represent introns (I), upstream (U) and downstream (D) regions. Continuous lines represent DNA fragments, which were used for sequence analysis. The discontinuous line in lambda 6 represent a region, which overlaps with lambda 8 and hence was not analyzed. The plasmid contains a PCR product, which bridges the gap between L3 and L6.

FIG. 16 presents the nucleotide sequence of the genomic region of the hpa gene. Exon sequences appear in upper case and intron sequences in lower case. The deduced amino acid, sequence of the exons is printed below the nucleotide sequence. Two predicted transcription start sites are shown in, bold.

FIG. 17 presents an alignment of the amino acid sequences of human heparanase, mouse and partial sequences of rat homologues. The human and the mouse sequences were determined by sequence analysis of the isolated cDNAs. The rat sequence is derived from two different EST clones, which represent two different regions (5' and 3') of the rat hpa cDNA. The human sequence and the amino acids in the mouse and rat homologues, which are identical to the human sequence, appear in bold.

FIG. 18 presents a heparanase Zoo blot. Ten ricrograms of genomic DNA from various sources were digested with EcoRI and separated on 0.77% agarose TBE gel. Following electrophoresis; the was gel treated with HCl and than with NaOH and the DNA fragments were downward transferred to a nylon membrane (Hybond N+, Amersham) with 0.4 N NaOH. The membrane was hybridized with a 1.6 Kb DNA probe that contained the entire hpa cDNA. Lane order: H Human; M Mouse; Rt Rat; P Pig; Cw Cow; Hr Horse; S Sheep; Rb Rabbit; D Dog; Ch Chicken; F Fish. Size markers (Lambda BsteII) are shown on the left

FIG. 19 demonstrates the secondary structure prediction for heparanase performed using the PHD server Profile network Prediction Heidelberg. H helix, E extended (beta strand), The glutamic acid predicted as the proton donor is marked by asterisk and the possible nucleophiles are underlined.

FIGS. 20a-b demonstrate the expression of heparanase by human endothelium. 20a RT-PCR. Total RNA isolated from ECGFstimulated proliferating human umbilical vein (HUVEC, lane 1) and bone marrow (TrHBMEC, lane 2) derived EC was analyzed by RTPCR for expression of the heparanase mRNA, using

human specific hpa primers amplifying a 564 bp cDNA (Vlodavsky, I. et al. Mammalian heparanase: gene cloning, expression and function, in tumor progression and metastasis. Nat Med 5, 793-802 (1999)) fragment. Lane 3, DNA molecular weight markers. 20b Immunohistochemistry. Immunostaining of tissue specimens was performed as described in the Examples section that follows. Positive staining is reddish-brown. Preferential staining of the heparanase protein is seen in the endothelium of capillaries and small sprouting vessels (arrows, left & right panels) as compared to little or no staining of endothelial cells in mature quiescent blood vessels (concave arrows, left & middle panels). Enhanced expression of the heparanase protein is seen in the neoplastic colonic epithelium. Original magnification is 200x (left and right panels) and 100x (middle panel).

FIGS. 21a-c demonstrate release of ECM-bound bFGF by recombinant heparanase, and bFGF accessory activity of HS degradation fragments released from EC-vs. ECM. 21a-b Release of ECM-bound bFGF. 21a ECM-coated wells of four-well plates were incubated (3-hours, 24 degrees C.) with 125I-bFGF as described in the Examples section that follows. The ECM was washed 3, times and incubated (3 hours, 37 degrees C.) with increasing concentrations; of recombinant heparanase. Released radioactivity is expressed as percent of the total ECM-bound 125I-bFGF. About 10;% of the ECM-bound 125I-bFGF was released in the absence of added heparanase. Each data point is the mean+-SD of triplicate wells. Where error-bars cannot be seen, SD is smaller than the symbol. 21a (inset) Release of sulfate labeled HS degradation fragments. Metabolically sulfate labeled ECM was incubated (3 hours, 37 degrees C., pH 6.0) with 0.2 mu g/ml recombinant heparanase. Sulfate labeled material released into the incubation medium was analyzed by gel filtration on Sepharose 6B. Labeled fragments eluted in fractions 15-35 (peak II) were 5-6 fold-smaller than intact HS side chains and were susceptible to deamination by ***nitrous*** ***acid*** (Vlodavsky, I. et al. Mammalian heparanase: gene cloning, expression and function in tumor progression and metastasis. Nat Med 5, 793-802 (1999)). 21b Release of endogenous ECM-resident bFGF by heparanase. Recombinant heparanase (0.5 g/ml) was incubated (4 hours, 37 degrees C.) with ECM coated 35-mm dishes in 1 ml heparanase reaction mixture. Aliquots of the incubation media were taken for quantitation of bFGF by ELISA as described in the Examples section that follows. Each data point is the mean+-S.D. of triplicate determinations. 21c Stimulation of bFGF induced DNA synthesis in BaF3 lymphoid cells by HS degradation fragments. Confluent bovine aortic EC cultured in 35-mm plates and their underlying ECM (as described in Gospodarowicz D. Moran J Braun D and Birdwell C 1976 Clonal growth of bovine vascular endothelial cells fibroblast growth factor as a survival agent. Proc. Natl. Acad. Sci. 73: 4120-4124) were incubated (4 hours, 37 degrees C., pH 6.5) with 0.1 mu g/ml recombinant heparanase. Aliquots (5-200 mu l) of the incubation media were then added to BaF3 cells seeded into 96 well plates in the presence of 5 ng/ml bFGF. 3H-thymidine (1 mu Ci/well) was added 48 hours after seeding and 6'hours ' later the cells were harvested and measured for 3H-thymidine incorporation. Each data point represents the mean+-S.D. of six culture wells. 21c (Inset) Release of sulfate labeled material from EC (open circles) vs. ECM (closed circles). In control plates, both the EC and ECM were first metabolically labeled with Na2(35S)O4. Sulfate labeled material released by heparanase (0.2 mu g/ml, 4 hours, 37 degrees C.) from EC and ECM was subjected to gel filtration.

FIGS. 22a-c demonstrate angiogenic response induced by Matrigel embedded with hpa vs. mock transfected Eb lymphoma cells. BALB/ c mice (n=5) were injected subcutaneously with 0.4 ml cold Matrigel premixed with 2x106 hpa- or mock-transfected Eb lymphoma cells. After 7 days, the mice were sacrified, and the Matrigel plugs were removed and photographed. Angiogenic response was then quantitated by measurement of the hemoglobin content as described in the Examples section that follows. 22a Representative Matrigel plugs containing hpa transfected (left) and mock transfected (right) Eb cells photographed in situ, prior to their removal out of their subcutaneous location in the mice. 22b Matrigel plugs containing heparanase producing (bottom) vs. control mock transfected (top) Eb cells. Shown are isolated Matrigel plugs removed from 10 different mice. 22c Hemoglobin content of Matrigel plugs (shown in FIG. 22b) containing hpa transfected (dark bar) vs. control mock transfected (empty bar) Eb cells. Represented is the mean+-SD (n#5, p=0.0089; unpaired t test).

FIGS. 23a-b demonstrate that topical administration of: active heparanase accelerate wound healing. 23a Full-thickness wounds were created with a circular 8 mm punch at the back of the mouse skin. Wound areas were

calculated after 7 days in control (1) or active heparanase-treated (2) mice and are shown as total area (23a) and percent (23b). Note the enhancement of wound healing upon exogenous application of heparanase. Data are statistically significant (P values equals 0.0023).

FIGS. 24a-d demonstrate an increase in granulation tissue cellularity upon heparanase treatment. Full-thickness wounds were created as described for FIGS. 24a-b. Wounds were left untreated (24a-b) or treated with heparanase for 7 days (24c-d). Wounds, including the underlying granulation tissue were formalin-fixed, paraffin-embedded and 5 micron sections were stained with hematoxilin-eosin. Note the increase in the granulation tissue cellularity upon heparanase treatment. Original magnifications: 24a and 24cx170; 24b and 24dx340.

FIGS. 25a-f demonstrate that heparanase treatment induces cellular proliferation and granulation tissue vascularization. Five micron sections from non-treated (25a, c and d) and heparanase-treated (25b, e and f) granulation tissues were stained for PCNA (25a-b and 25d-e) and for PECAM-1 (25c, f). Note the increase in PCNA-positive cells and PECAM-1 positive blood vessels structures upon heparanase treatment. Original magnifications: 25a-cX 170, 25d-fX 340.

FIGS. 26a-f demonstrates, that heparanase expression is restricted to differentiated keratinocytes in mouse skin tissue. Five micron skin tissue sections were stained for PCNA (26a, d) and heparanase (26b-c, e). Negative control (no primary antibody) is shown in 26f. Note intense PCNA staining at the basal epidermal cell layer (26a, d) while heparanase mainly stain the outer most, keratinocytes, cell layer (26b, e) and the cells composing the hair follicle (26c). In the latter case, nuclear staining is observed.

FIGS. 27a-d demonstrate expression of heparanase in human skin. 27a cultures of HaCat keratinocytes cell line immunostained with anti-heparanase monoclonal antibody (HP-92). 27b heparanase activity in intact cells and in ***extracts*** of HaCat cells, in an ECM-assay. 27c and d immuno-staining of normal skin tissue with HP-92.

FIG. 28 demonstrates stimulation of angiogenesis by heparanase in rat eye model. The central cornea of rats' eyes was scraped with a surgical knife. The right eye of each rat was then treated with heparanase, 50 mu l drop (1 mg/ml) of purified recombinant human P50 heparanase, three times a day. The left eye served as a control and was treated with Lyeteers. Vascularization and epithelialization were evaluated following closure of the corneal lesion. Heparanase treated eyes exhibited vascularization of the cornea, as well as increased vascularization in the iris. Normal, minor vascularization of the iris and non vascular appearance of the cornea were observed in the controls

FIG. 29 demonstrates cornea sections of heparanase treated eye as compared to control, Lyeteers treated eyes. Control eyes demonstrate healing of the epithelia which is accompanied by a normal organized structure of the cornea. Heparanase treatment resulted in growth of blood vessels into the cornea (arrows), followed by a massive infiltration of lymphocytes. Vascularization associated inflammatory reaction interfered with corneal healing, as demonstrated by a disorganized structure of the cornea.

FIGS. 30a-e demonstrate that skin tissue morphology is impaired under diabetic conditions. Skin sections from normal (30a, 30d) and streptozotocin-induced diabetic (b, e) rats were hematoxilin-eosin stained (30a, 30b) or immunostained with antiheparanase antibodies (30d, 30e). Measurements from 10 control or diabetic different rats are shown in (30c). Note a dramatic decrease in the skin tissue thickness and reduced heparanase expression under diabetic conditions.

FIGS. 31a-f demonstrate heparanase expression in the wound granulation tissue. Full-thickness wounds were generated by 8 mm punch at the back of rat skin. Seven days later the wounds, including the newly formed granulation tissue, were harvested, formalin-fixed and paraffin-embedded. Five microns sectionswere stained for heparanase (31a-c), or double stained for heparanase (red) and SMA (green)(31d-f). Note heparanase expression in the granulation tissue (31a) and at the lumenfacing areas of endothelial cells lining blood vessels (31e, 31f). Original magnifications: a x4, b x10, c-fx40.

FIG. 32 demonstrates that heparanase accelerates wound healing in streptozotocin-induced rat diabetic. Four 8 mm fullthickness punches were created at the back of normal, nondiabetic (Nor), or diabetic rats. Wounds were treated with saline (Nor, Con), heparanase (Hep, 1; mu g/wound) or PDGF (0.5 mu g/wound) immediately following wounding, four hours later, and three additional times during the following day, at 4 hours intervals. Seven days after wounding, wounds were harvested, fixed

and wound closure was evaluated under low power magnification of hematoxilin-eosin stained sections. Three animals were included min each group to yield 12 wounds for each treatment. Note improved wound healing upon heparanase treatment, similar to PDGF effect.

FIGS. 33a-b demonstrate that heparanase accelerates wound healing under ischemic conditions. FIG. 33a is a schematic representation of the flap/punch ischemic wound model. Two longitudinal incisions, each 6 cm in length, were connected at the caudal end with a third, 3 cm, incision across the midline. The flap was elevated to the base of the carnial pedicle, replaced in its: bed and secured with sutures. Two 8 mm punches were generated in the flap 3 cm from the carnial end. FIG. 33b Wounds were treated with saline (Con), active heparanase (p45, 1 mu g/wound), the heparanase precursor (p60, 5 mu g/wound) and PDGF (0.5 mu g/wound) immediately after wounding, 4 hours later and three more times, 4 hours apart, the next day (a total of 5 application, each at a volume of 50 mu l). Longitude incisions were treated once just prior to clipping. Wounds closure was evaluated 10 days following wounding by histological examination. P45 as well: as, p60 heparanases significantly improved wound closure (p values are 0.03 and 0.016 for p45 and p60, respectively). Five rats were included in each group, and two wounds were created at each flap to yield a total of 10 wounds.

FIG. 34 demonstrates that heparanase induces reepithalialization of incisional wounds. Typical histological examination of control (left) and heparanase (p45) treated incisional wounds from the flap described in FIG. 33a-b is shown Measurements of 10 incisions from control and V heparanase treated incisions are shown graphically. Note a robust increase in the epithelial layer thickness upon heparanase treatment.

FIG. 35 demonstrates % that heparanase treatment induces the recruitment of pericytes into blood vessels. Untreated (Con) and heparanase-treated (Hep) wound sections from the ischemic model were immunostained with anti-SMA antibodies. Representative photomicrographs are shown on the left and graphical evaluation of 10 different wounds, and at least 3 different fields in each wound, is shown on the right. Note the dramatic recruitment of SMA-positive pericytes into blood vessels upon heparanase treatment.! OF 17 IFIPAT COPYRIGHT 2006 IFI on STN

AB Methods and compositions for inducing and/or accelerating wound healing and/or angiogenesis via the catalytic activity of heparanase are disclosed.

L3 ANSWER 3 OF 17 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 2005-22594 BIOTECHDS
TI Novel isolated Antrodia camphorata protein ACA1, useful for preparing chemical composition having immunomodulatory activity;
Antrodia camphorata protein isolation for use in disease therapy and cell proliferation
AU SHEU F; HSIEH K; CHIEN P; TSAO C; CHIN K
PA CHIEN P
PI US 2005164931 28 Jul 2005
AI US 2004-15078 18 Dec 2004
PRAI TW 2003-136095 19 Dec 2003; TW 2003-136095 19 Dec 2003
DT Patent
LA English
OS WPI: 2005-521436 [53]
AB DERWENT ABSTRACT:
NOVELTY - An isolated Antrodia camphorata protein ACA1 (I) having a fully defined 118 amino acid (SEQ ID No. 2) sequence given in the specification, or having an amino acid sequence being at least 90% similar to SEQ ID No. 2, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a chemical composition (II) comprising (I) as an active ingredient, where (II) has immunomodulatory activity; and (2) an isolated polynucleotide comprising the nucleotide sequence of a fully defined 354 nucleotide (SEQ ID No. 1) sequence given in the specification.

BIOTECHNOLOGY - Preferred Protein: (I) further possesses immunomodulatory activity.

ACTIVITY - Cytostatic; Antiinflammatory; Hepatotropic; Antibacterial; Virucide.

MECHANISM OF ACTION - Immunomodulator (claimed); Promotes lymphocyte proliferation; Activates macrophages; Enhances production of TNF-alpha and nitric oxide by macrophages. In vitro analysis of the efficacy of ACA1 protein in activating macrophages was carried out as follows. RAW 264.7 macrophages were cultivated in 96-well plate at 37degreesC for 24

hours, in a 5% carbon dioxide incubator. Then, ACA1 protein was added into the well at a concentration of 0, 5, 10, 20, 40, 80 and 160 micrograms/ml, and cultivation of the cell was carried out for 20 hours at.37degreesC, in a carbon dioxide incubator (5%). The cell culture (100 mul) was added with Griess reagent (100 mul) comprising N-(1-napthyl)ethylenediamine hydrochloride (0.05%), sulfanilamide (0.5%) and orthophosphoric acid (2.5%) in acidity solution, to allow the reaction of ***nitrous*** ***acid*** of the culture with Griess reagent to become purple azo dye. The nitrite concentration of sample was determined. The result indicated activation of macrophage by ACA1 (20 micrograms/ml) and production of nitric oxide (capable of killing bacteria). The nitrite yield was 13.2 muM.

USE - (I) is useful for preparing a chemical composition having immunomodulatory activity (claimed). (I) is useful for treating hepato tumor and uterus tumor, reducing liver fibrosis, promoting lymphocyte proliferation, and for its antihepatitis B ***virus*** activity. (I) is useful for activating RAW 264.7 macrophage and enhancing the production of TNF-alpha and nitric oxide by RAW 264.7 macrophages, where the nitric oxide is useful for killing pathogenic bacteria.

EXAMPLE - The ferment and mycelium in Antrodia camphorata ferment were separated by centrifugation. The mycelium was washed 3 times using water and then extra water was removed from the A.camphorata ferment by centrifugation. The mycelium was transferred into an ***extraction*** buffer comprising beta-mercaptoethanol (in v/v%) (0.1) in glacial acetic acid (5) and ground to a thick liquid. The resultant thick liquid was centrifuged. Ammonium sulfate was slowly added to the superstrata of the thick liquid and the mixture was stirred overnight. The precipitates were dialyzed against Tris-hydrochloric acid buffer, centrifuged and the superstrata was collected as crude protein liquid of A.camphorata ammonium sulfate. The crude protein liquid was purified in a diethylaminoethyl (DEAE)-52 cellulose column.(22 pages)

L3 ANSWER 4 OF 17 IFIPAT COPYRIGHT 2006 IFI on STN DUPLICATE 1
AN 10668457 IFIPAT;IFIUDB;IFICDB
TI METHODS, COMPOSITIONS AND KITS FOR BIOMARKER EXTRACTION
INF Debad; Jeff D., Gaithersburg, MD, US
Ly; Cindy V., Houston, TX, US
IN Debad Jeff D; Ly Cindy V
PAF Unassigned
PA Unassigned Or Assigned To Individual (68000)
PPA Meso Scale Technology (Probable)
AG NIXON & VANDERHYE, PC, 1100 N GLEBE ROAD, 8TH FLOOR, ARLINGTON, VA, 22201-4714, US
PI US 2004175695 A1 20040909
AI US 2003-736899 20031217
PRAI US 2002-436591P 20021226 (Provisional)
FI US 2004175695 20040909
DT Utility; Patent Application - First Publication
FS CHEMICAL
APPLICATION
PARN This application claims priority to U.S. Provisional Application No. 60/435,591, filed Dec. 26, 2002, which is incorporated herein by reference.
CLMN 131
OF 17 IFIPAT COPYRIGHT 2006 IFI on STN DUPLICATE 1
AB The present invention is directed to methods for extracting markers from biological samples, and to systems, devices, kits and reagents for use in such methods. The invention is also to methods, kits, reagents and compositions for measuring a plurality of different organism types in a sample. One of the specific advantages of the present invention is the ability to simultaneously extract more than one microorganism or viral particle marker in one volume from a single sample containing a complex biological matrix.

L3 ANSWER 5 OF 17 IFIPAT COPYRIGHT 2006 IFI on STN
AN 10639269 IFIPAT;IFIUDB;IFICDB
TI THERAPEUTIC AND COSMETIC USES OF HEPARANASES
INF Feinstein; Elena, Rehovot, IL
Ilan; Neta, Rehovot, IL
Pecker; Iris, Rishon LeZion, IL
Vlodavsky; Israel, Mevaseret Zion, IL
Yacoby-Zeevi; Oron, Moshav Bizaron, IL

IN Feinstein Elena (IL); Ilan Neta (IL); Pecker Iris (IL); Vlodavsky Israel (IL); Yacoby-Zeevi Oron (IL)
PAF Unassigned
PA Unassigned Or Assigned To Individual (68000)
AG SOL SHEINBEIN;c/o ANTHONY CASTORINA, SUITE 207, 2001 JEFFERSON DAVIS HIGHWAY, ARLINGTON, VA, 22202, US
PI US 2004146497 A1 20040729
AI US 2004-781758 20040220
RLI US 1999-258892 19990301 CONTINUATION ABANDONED
US 2001-776874 20010206 CONTINUATION PENDING
US 2003-341582 20030114 CONTINUATION PENDING
US 1997-922170 19970902 CONTINUATION-IN-PART 5968822
WO 1998-US17954 19980831 CONTINUATION-IN-PART PENDING
WO 2001-IL830 20010905 CONTINUATION-IN-PART
US 2001-988113 20011119 CONTINUATION-IN-PART PENDING
PRAI US 2000-231551P 20000911 (Provisional)
US 2000-244593P 20001101 (Provisional)
FI US 2004146497 20040729
US 5968822
DT Utility; Patent Application - First Publication
FS CHEMICAL
APPLICATION
CLMN 84
GI 35 Figure(s).

FIG. 1 presents nucleotide sequence and deduced amino acid sequence of hpa cDNA. A single nucleotide difference at position 799 (A to T) between the EST (Expressed Sequence Tag) and the PCR amplified cDNA (reverse transcribed RNA) and the resulting amino acid substitution (Tyr to Phe) are indicated above and below the substituted unit, respectively. Cysteine residues and the poly adenylation consensus sequence are underlined. The asterisk denotes the stop codon TGA.

FIG. 2 demonstrates degradation of soluble sulfate labeled HSPG substrate by lysates of High Five cells infected with pFhpa2 ***virus*** . Lysates of High Five cells that were infected with pFhpa2 ***virus*** (small-circle) or control pF2 ***virus*** (.squ.) were incubated (18 h, 37 degrees C.) with sulfate labeled ECMderived soluble HSPG (peak I). The incubation medium was then subjected to gel filtration on Sepharose 6B. Low molecular weight HS degradation fragments (peak II) were produced only during incubation with the pFhpa2 infected cells, but there was no degradation of the HSPG substrate (open-diamond) by lysates of pF2 infected cells.

FIGS. 3a-b demonstrate degradation of soluble sulfate labeled HSPG substrate by the culture medium of pFhpa2 and pFhpa4 infected cells. Culture media of High Five cells infected with pFhpa2 (3a) or pFhpa4 (3b) ***viruses*** (small-circle), or with control ***viruses*** (.squ.) were incubated (18 h, 37 degrees C.) with sulfate labeled ECM-derived soluble HSPG (peak I, opendiamond). The incubation media were then subjected to gel filtration on Sepharose 6B. Low molecular weight HS degradation fragments (peak II) were produced only during incubation with the hpa gene containing ***viruses*** . There was no degradation of the HSPG substrate by the culture medium of cells infected with control ***viruses*** .

FIG. 4 presents size fractionation of heparanase activity expressed by pFhpa2 infected cells. Culture medium of pFhpa2 infected High Five cells was applied onto a 50 kDa cut-off membrane. Heparanase activity (conversion of the peak I substrate, (open-diamond) into peak II HS degradation fragments) was found in the high (>50 kDa) (small-circle), but not low (<50 kDa) (composite) molecular weight compartment.

FIGS. 5a-b demonstrate the effect of heparin on heparanase activity expressed by pFhpa2 and pFhpa4 infected High Five cells. Culture media of pFhpa2 (5a) and pFhpa4 (5b) infected High Five cells were incubated (18 h, 37 degrees C.) with sulfate labeled ECM-derived soluble HSPG (peak I, open-diamond) in the absence (small-circle) or presence (Delta) of 10 mu g/ml heparin. Production of low molecular weight HS degradation fragments was completely abolished in the presence of heparin, a potent inhibitor of heparanase activity (6, 7).

FIGS. 6a-b demonstrate degradation of sulfate labeled intact ECM by ***virus*** infected High Five and Sf21 cells. High Five (6a) and Sf21 (6b) cells were plated on sulfate labeled ECM and infected (48 h, 28 degrees C.) with pFhpa4 (small-circle) or control pF1 (.squ.) ***viruses*** . Control non-infected Sf21 cells (R) were plated on the labeled ECM as well. The pH of the cultured medium was adjusted to

6.0-6.2 followed by 24 h incubation at 37 degrees C. Sulfate labeled material released into the incubation medium was analyzed by gel filtration on Sepharose 6B. HS degradation fragments were produced only by cells infected with the hpa containing ***virus*** .

FIGS. 7a-b demonstrate degradation of sulfate labeled intact ECM by ***virus*** infected cells. High Five (7a) and Sf21 (7b) cells were plated on sulfate labeled ECM and infected (48 h, 28 degrees C.) with pFhpa4 (small-circle) or control pF1 (.squ.) ***viruses*** . Control non-infected Sf21 cells (R) were plate on labeled ECM as well. The pH of the cultured medium was adjusted to 6.0-6.2, followed by 48 h incubation at 28 degrees C. Sulfate labeled degradation fragments released into the incubation medium was analyzed by gel filtration on Sepharose 6B. HS degradation fragments were produced only by cells infected with the hpa containing ***virus*** .

FIGS. 8a-b demonstrate degradation of sulfate labeled intact ECM by the culture medium of pFhpa4 infected cells. Culture media of High Five (8a) and Sf21 (8b) cells that were infected with pFhpa4 (small-circle) or control pF1 (.squ.) ***viruses*** were incubated (48 h, 37 degrees C., pH 6.0) with intact sulfate labeled ECM. The ECM was also incubated with the culture medium of control non-infected Sf21 cells (R). Sulfate labeled material released into the reaction mixture was subjected to gel filtration analysis. Heparanase activity was detected only in the culture medium of pFhpa4 infected cells.

FIGS. 9a-b demonstrate the effect of heparin on heparanase activity in the culture medium of pFhpa4 infected cells. Sulfate labeled ECM was incubated (24 h, 37 degrees C., pH 6.0) with culture medium of pFhpa4 infected High Five (9a) and Sf21 (9b) cells in the absence (small-circle) or presence (V) of 10 mu g/ml heparin. Sulfate labeled material released into the incubation medium was subjected to gel filtration on Sepharose 6B. Heparanase activity (production of peak II HS degradation fragments) was completely inhibited in the presence of heparin.

FIGS. 10a-b demonstrate purification of recombinant heparanase on heparin-Sepharose. Culture medium of Sf21 cells infected with pFhpa4 ***virus*** was subjected to heparin-Sepharose chromatography. Elution of fractions was performed with 0.35-2 M NaCl gradient (open-diamond). Heparanase activity in the eluted fractions is demonstrated in FIG. 10a (small-circle). Fractions 15-28 were subjected to 15% SDS-polyacrylamide gel electrophoresis followed by silver nitrate staining. A correlation is demonstrated between a major protein band (MW63, 000) in fractions 19-24 and heparanase activity.

FIGS. 11a-b demonstrate purification of recombinant heparanase on a Superdex 75 gel filtration column. Active fractions eluted from heparin-Sepharose (FIG. 10a) were pooled, concentrated and applied onto Superdex 75 FPLC column. Fractions were collected and aliquots of each fraction were tested for heparanase activity (c, FIG. 11a) and analyzed by SDS-polyacrylamide gel electrophoresis followed by silver nitrate staining (FIG. 11b). A correlation is seen between the appearance of a major protein band (MW63,000) in fractions 4-7 and heparanase activity.

FIGS. 12a-e demonstrate expression of the hpa gene by RT-PCR with total RNA from human embryonal tissues (12a), human extraembryonal tissues (12b) and cell lines from different origins (12c-e). RT-PCR products using hpa specific primers (I), primers for GAPDH housekeeping gene (II), and control reactions without reverse transcriptase demonstrating absence of genomic DNA or other contamination in RNA samples (III). M-DNA molecular weight marker VI (Boehringer Mannheim). For 12a: lane 1 neutrophil cells (adult), lane 2 muscle, lane 3 thymus, lane 4 heart, lane 5 adrenal. For 12b: lane 1 kidney, lane 2 placenta (8 weeks), lane 3 placenta (11 weeks), lanes 4-7-mole (complete hydatidiform mole), lane 8 cytotrophoblast cells (freshly isolated), lane 9 cytotrophoblast cells (1.5 h in vitro), lane 10 cytotrophoblast cells (6 h in vitro), lane 11 cytotrophoblast cells (18 h in vitro), lane 12 cytotrophoblast cells (48 h in vitro). For 12c: lane 1 JAR bladder cell line, lane 2 NCITT testicular tumor cell line, lane 3 SW-480 human hepatoma cell line, lane 4 HTR (cytotrophoblasts transformed by SV40), lane 5 HPTLP-I hepatocellular carcinoma cell line, lane 6 EJ-28 bladder carcinoma cell line. For 12d: lane 1 SK-hep-1 human hepatoma cell line, lane 2 DAMI human megakaryocytic cell line, lane 3 DAMI cell line+PMA, lane 4 CHRF cell line+PMA, lane 5 CHRF cell line. For 12e: lane 1 ABAE bovine aortic endothelial cells, lane 2-1063 human ovarian cell line, lane 3 human breast carcinoma MDA435 cell line, lane 4 human breast carcinoma MDA231 cell line.

FIG. 13 presents a comparison between nucleotide sequences of the human

hpa and a mouse EST cDNA fragment (SEQ ID NO: 12) which is 80% homologous to the 3' end (starting at nucleotide 1066 of SEQ ID NO:9) of the human hpa. The aligned termination codons are underlined.

FIG. 14 demonstrates the chromosomal localization of the hpa gene. PCR products of DNA derived from somatic cell hybrids and of genomic DNA of hamster, mouse and human of were separated on 0.7% agarose gel following amplification with hpa specific primers. Lane 1 Lambda DNA digested with BstEII, lane 2 no DNA control, lanes 3-29, PCR amplification products. Lanes 3-5 human, mouse and hamster genomic DNA, respectively. Lanes 6-29, human monochromosomal somatic cell hybrids representing chromosomes 1-22 and X and Y, respectively. Lane 30 Lambda DNA digested with BstEII. An amplification product of approximately 2.8 Kb is observed only in lanes 5 and 9, representing human genomic DNA and DNA derived from cell hybrid carrying human chromosome 4, respectively. These results demonstrate that the hpa gene is localized in human chromosome 4.

FIG. 15 demonstrates the genomic exon-intron structure of the human hpa locus (top) and the relative positions of the lambda clones used as sequencing templates to sequence the locus (below). The vertical rectangles represent exons (E) and the horizontal lines therebetween represent introns (I), upstream (U) and downstream (D) regions. Continuous lines represent DNA fragments, which were used for sequence analysis. The discontinuous line in lambda 6 represent a region, which overlaps with lambda 8 and hence was not analyzed. The plasmid contains a PCR product, which bridges the gap between L3 and L6.

FIG. 16 presents the nucleotide sequence of the genomic region of the hpa gene. Exon sequences appear in upper case and intron sequences in lower case. The deduced amino acid sequence of the exons is printed below the nucleotide sequence. Two predicted transcription start sites are shown in bold.

FIG. 17 presents an alignment of the amino acid sequences of human heparanase, mouse and partial sequences of rat homologues. The human and the mouse sequences were determined by sequence analysis of the isolated cDNAs. The rat sequence is derived from two different EST clones, which represent two different regions (5' and 3') of the rat hpa cDNA. The human sequence and the amino acids in the mouse and rat homologues, which are identical to the human sequence, appear in bold.

FIG. 18 presents a heparanase Zoo blot. Ten micrograms of genomic DNA from various sources were digested with EcoRI and separated on 0.7% agarose TBE gel. Following electrophoresis, the was gel treated with HCl and than with NaOH and the DNA fragments were downward transferred to a nylon membrane (Hybond N+, Amersham) with 0.4 N NaOH. The membrane was hybridized with a 1.6 Kb DNA probe that contained the entire hpa cDNA. Lane order: H Human; M Mouse; Rt Rat; P Pig; Cw Cow; Hr Horse; S Sheep; Rb Rabbit; D Dog; Ch Chicken; F Fish. Size markers (Lambda BsteII) are shown on the left

FIG. 19 demonstrates the secondary structure prediction for heparanase performed using the PHD server Profile network Prediction Heidelberg. H helix, E extended (beta strand), The glutamic acid predicted as the proton donor is marked by asterisk and the possible nucleophiles are underlined.

FIGS. 20a-b demonstrate the expression of heparanase by human endothelium. 20a RT-PCR. Total RNA isolated from ECGFstimulated proliferating human umbilical vein (HUVEC, lane 1) and bone marrow (TrHBMEC, lane 2) derived EC was analyzed by RTPCR for expression of the heparanase mRNA, using human specific hpa primers amplifying a 564 bp cDNA (Vlodavsky, I. et al. Mammalian heparanase: gene cloning, expression and function in tumor progression and metastasis. Nat Med 5., 793-802 (1999)) fragment. Lane 3, DNA molecular weight markers. 20b Immunohistochemistry. Immunostaining of tissue specimens was performed as described in the Examples section that follows. Positive staining is reddish-brown. Preferential staining of the heparanase protein is seen in the endothelium of capillaries and small sprouting vessels (arrows, left & right panels) as compared to little or no staining of endothelial cells in mature quiescent blood vessels (concave arrows, left & middle panels). Enhanced expression of the heparanase protein is seen in the neoplastic colonic epithelium. Original magnification is 200x (left and right panels) and 100x (middle panel).

FIGS. 21a-c demonstrate release of ECM-bound bFGF by recombinant heparanase, and bFGF accessory activity of HS degradation fragments released from EC vs. ECM. 21a-b Release of ECM-bound bFGF. 21a ECM-coated wells of four-well plates were incubated (3 hours, 24 degrees C.) with 125I-bFGF as described in the Examples section that follows. The ECM was washed 3 times and incubated (3 hours, 37 degrees C.) with increasing

concentrations of recombinant heparanase. Released radioactivity is expressed as percent of the total ECM-bound 125I-bFGF. About 10% of the ECM-bound 125I-bFGF was released in the absence of added heparanase. Each data point is the mean+SD of triplicate wells. Where error bars cannot be seen, SD is smaller than the symbol. 21a (inset) Release of sulfate labeled HS degradation fragments. Metabolically sulfate labeled ECM was incubated (3 hours, 37 degrees C., pH 6.0) with 0.2 mu g/ml recombinant heparanase. Sulfate labeled material released into the incubation medium was analyzed by gel filtration on Sepharose 6B. Labeled fragments eluted in fractions 15-35 (peak II) were 5-6 fold smaller than intact HS side chains and were susceptible to deamination by ***nitrous*** ***acid*** (Vlodavsky, I. et al. Mammalian heparanase: gene cloning, expression and function in tumor progression and metastasis. Nat Med 5, 793-802 (1999)). 21b Release of endogenous ECM-resident bFGF by heparanase. Recombinant heparanase (0.5 mu g/ml) was incubated (4 hours, 37 degrees C.) with ECM coated 35-mm dishes in 1 ml heparanase reaction mixture. Aliquots of the incubation media were taken for quantitation of bFGF by ELISA as described in the Examples section that follows. Each data point is the mean+-S.D. of triplicate determinations. 21c Stimulation of bFGF induced DNA synthesis in BaF3 lymphoid cells by HS degradation fragments. Confluent bovine aortic EC cultured in 35-mm plates and their underlying ECM (as described in Gospodarowicz D. Moran J Braun D and Birdwell C 1976 Clonal growth of bovine vascular endothelial cells: fibroblast growth factor as a survival agent. Proc. Natl. Acad. Sci. 73: 4120-4124) were incubated (4 hours, 37 degrees C., pH 6.5) with 0.1 mu g/ml recombinant heparanase. Aliquots (5-200 mu l) of the incubation media were then added to BaF3 cells seeded into 96 well plates in the presence of 5 ng/ml bFGF. 3H-thymidine (1 mu Ci/well) was added 48 hours after seeding and 6 hours later the cells were harvested and measured for 3H-thymidine incorporation. Each data point represents the mean+-S.D. of six culture wells. 21c (Inset) Release of sulfate labeled material from EC (open circles) vs. ECM (closed circles). In control plates, both the EC and ECM were first metabolically labeled with Na2(35S)O4. Sulfate labeled material released by heparanase (0.2 mu g/ml, 4 hours, 37 degrees C.) from EC and ECM was subjected to gel filtration.

FIGS. 22a-c demonstrate angiogenic response induced by Matrigel embedded with hpa vs. mock transfected Eb lymphoma cells. BALB/ c mice (n=5) were injected subcutaneously with 0.4 ml cold Matrigel premixed with 2x106 hpa- or mock-transfected Eb lymphoma cells. After 7 days, the mice were sacrified, and the Matrigel plugs were removed and photographed.

FIGS. 23a-b demonstrate that topical administration of active heparanase accelerate wound healing. 23a Full-thickness wounds were created with a circular 8 mm punch at the back of the mouse skin. Wound areas were calculated after 7 days in control (1) or active heparanase-treated (2) mice and are shown as total area (23a) and percent (23b). Note the enhancement of wound healing upon exogenous application of heparanase. Data are statistically significant (P values equals 0.0023).

FIGS. 24a-d demonstrate an increase in granulation tissue cellularity upon heparanase treatment. Full-thickness wounds were created as described for FIGS. 24a-b. Wounds were left untreated (24a-b) or treated with heparanase for 7 days (24c-d). Wounds, including the underlying granulation tissue were formalin-fixed, paraffin-embedded and 5 micron sections were stained with hematoxilin-eosin. Note the increase in the granulation tissue cellularity upon heparanase treatment. Original magnifications: 24a and 24cx170; 24b and 24dx340.

FIGS. 25a-f demonstrate that heparanase treatment induces cellular proliferation and granulation tissue vascularization. Five micron sections from non-treated (25a, c and d) and heparanase-treated (25b, e and f) granulation tissues were stained for PCNA (25a-b and 25d-e) and for PECAM-1 (25c, f). Note the increase in PCNA-positive cells and PECAM-1 positive blood vessels structures upon heparanase treatment. Original magnifications: 25a-cx170, 25d-fx340.

FIGS. 26a-f demonstrates that heparanase expression is restricted to differentiated keratinocytes in mouse skin tissue. Five micron skin tissue sections were stained for PCNA (26a, d) and heparanase (26b-c, e). Negative control (no primary antibody) is shown in 26f. Note intense PCNA staining at the basal epidermal cell layer (26a, d) while heparanase mainly stain the outer most, keratinocytes, cell layer (26b, e) and the cells composing the hair follicle (26c). In the latter case, nuclear staining is observed.

FIGS. 27a-d demonstrate expression of heparanase in human skin. 27a cultures of HaCat keratinocytes cell line immunostained with

anti-heparanase monoclonal antibody (HP-92). 27b heparanase activity in intact cells and in ***extracts*** of HaCat cells, in an ECM-assay. 27c and d immuno-staining of normal skin tissue with HP-92.

FIG. 28 demonstrates stimulation of angiogenesis by heparanase in rat eye model. The central cornea of rats' eyes was scraped with a surgical knife. The right eye of each rat was then treated with heparanase, 50 mu l drop (1 mg/ml) of purified recombinant human P50 heparanase, three times a day. The left eye served as a control and was treated with Lyeteers. Vascularization and epithelialization were evaluated following closure of the corneal lesion. Heparanase treated eyes exhibited vascularization of the cornea, as well as increased vascularization in the iris. Normal, minor vascularization of the iris and non vascular appearance of the cornea were observed in the controls

FIG. 29 demonstrates cornea sections of heparanase treated eye as compared to control, Lyeteers treated eyes. Control eyes demonstrate healing of the epithelia which is accompanied by a normal organized structure of the cornea. Heparanase treatment resulted in growth of blood vessels into the cornea (arrows), followed by a massive infiltration of lymphocytes. Vascularization associated inflammatory reaction interfered with corneal healing, as demonstrated by a disorganized structure of the cornea.

FIGS. 30a-e demonstrate that skin tissue morphology is impaired under diabetic conditions. Skin sections from normal (30a, 30d) and streptozotocin-induced diabetic (b, e) rats were hematoxilin-eosin stained (30a, 30b) or immunostained with antiheparanase antibodies (30d, 30e). Measurements from 10 control or diabetic different rats are shown in (30c). Note a dramatic decrease in the skin tissue thickness and reduced heparanase expression under diabetic conditions.

FIGS. 31a-f demonstrate heparanase expression in the wound granulation tissue. Full-thickness wounds were generated by 8 mm punch at the back of rat skin. Seven days later the wounds, including the newly formed granulation tissue, were harvested, formalin-fixed and paraffin-embedded. Five microns sections were stained for heparanase (31a-c), or double stained for heparanase (red) and SMA (green)(31d-f). Note heparanase expression in the granulation tissue (31a) and at the lumenfacing areas of endothelial cells lining blood vessels (31e, 31f). Original magnifications: ax4, bx10, c-fx40.

FIG. 32 demonstrates that heparanase accelerates wound healing in streptozotocin-induced rat diabetic. Four 8 mm fullthickness punches were created at the back of normal, nondiabetic (Nor), or diabetic rats. Wounds were treated with saline (Nor, Con), heparanase (Hep, 1 mu g/wound) or PDGF (0.5 mu g/wound) immediately following wounding, four hours later, and three additional times during the following day, at 4 hours intervals. Seven days after wounding, wounds were harvested, fixed and wound closure was evaluated under low power magnification of hematoxilin-eosin stained sections. Three animals were included in each group to yield 12 wounds for each treatment. Note improved wound healing upon heparanase treatment, similar to PDGF effect.

FIGS. 33a-b demonstrate that heparanase accelerates wound healing under ischemic conditions. FIG. 33a is a schematic representation of the flap/punch ischemic wound model. Two longitudinal incisions, each 6 cm in length, were connected at the caudal end with a third, 3 cm, incision across the midline. The flap was elevated to the base of the carnial pedicle, replaced in its bed and secured with sutures. Two 8 mm punches were generated in the flap 3 cm from the carnial end. FIG. 33b Wounds were treated with saline (Con), active heparanase (p45, 1 mu g/wound), the heparanase precursor (p60, 5 mu g/wound) and PDGF (0.5 mu g/wound) immediately after wounding, 4 hours later and three more times, 4 hours apart, the next day (a total of 5 application, each at a volume of 50 mu l). Longitude incisions were treated once just prior to clipping. Wounds closure was evaluated 10 days following wounding by histological examination. P45 as well as p60 heparanases significantly improved wound closure (p values are 0.03 and 0.016 for p45 and p60, respectively). Five rats were included in each group, and two wounds were created at each flap to yield a total of 10 wounds.

FIG. 34 demonstrates that heparanase induces reepithalialization of incisional wounds. Typical histological examination of control (left) and heparanase (p45)-treated incisional wounds from the flap described in FIGS. 33a-b is shown. Measurements of 10 incisions from control and heparanase treated incisions are shown graphically. Note a robust increase in the epithelial layer thickness upon heparanase treatment.

FIG. 35 demonstrates that heparanase treatment induces the recruitment of pericytes into blood vessels. Untreated (Con) and heparanase-treated

(Hep) wound sections from the ischemic model were immunostained with anti-SMA antibodies. Representative photomicrographs are shown on the left and graphical evaluation of 10 different wounds, and at least 3 different fields in each wound, is shown on the right. Note the dramatic recruitment of SMA-positive pericytes into blood vessels upon heparanase treatment. !

AB Methods and compositions for inducing and/or accelerating wound healing and/or angiogenesis via the catalytic activity of heparanase are disclosed.

L3 ANSWER 6 OF 17 USPATFULL on STN
AN 2004:18446 USPATFULL
TI Medication combination for hepatoma and pancreatic cancer and preparation protocol
IN Lee, Chien-Yung, Kaohsiung Hsien, TAIWAN, PROVINCE OF CHINA
PI US 2004013748 A1 20040122
AI US 2002-252123 A1 20020923 (10)
PRAI TW 2002-2124451 20020626
DT Utility
FS APPLICATION
LREP BROWDY AND NEIMARK, P.L.L.C., 624 NINTH STREET, NW, SUITE 300, WASHINGTON, DC, 20001-5303
CLMN Number of Claims: 8
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 4055
AB This invention discloses a medication combination, consisting of Baizhu, Danggui, Hanxincao, Huotanmucao, Ainaxiang, Shuodiao, Malan, Ludou, Canger, Daqinggen, Banbianlian, Xingren, Nuzhenzi, Qianhu, Jiatonghao, Yinchenhao, Yujin, Zhishi, Banxia, and Fuling for treating hepatoma and pancreatic cancer, modifies the conventional extraction procedure and is characterized by the inclusion of Poria, which possesses an anti-tumor ability, as the excipient. This medication combination is based on a novel and continuous theory for cancer therapy and traditional Chinese medicine theories. It has made use of nature and characteristics of those abovementioned medications to lead and change the environments for cancer growth inside the patients' bodies, and then to result in the discomfort and revulsion of cancer cells in such environments. In another word, this medication combination alters the cancer environment via pharmacology and exerts a certain extent of anti-tumor effect on the primary or metastatic hepatoma and pancreatic cancer.

L3 ANSWER 7 OF 17 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
AN 2004-553191 [53] WPIDS
DNN N2004-437690 DNC C2004-202403
TI Measuring different organisms in a sample, useful for diagnosing e.g., pharingytis, comprises contacting the sample with an extraction reagent comprising nitrous acid and measuring markers of the organisms.
DC B04 D16 S03
IN DEBAD, J D; LY, C V; LY, C
PA (DEBA-I) DEBAD J D; (LYCV-I) LY C V; (MESO-N) MESO SCALE TECHNOLOGIES LLC
CYC 108
PI WO 2004059280 A2 20040715 (200453)* EN 56
RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
US 2004175695 A1 20040909 (200459)
AU 2003293562 A1 20040722 (200476)
EP 1604183 A2 20051214 (200582) EN
R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LT LU LV MC MK NL PT RO SE SI SK TR
ADT WO 2004059280 A2 WO 2003-US39938 20031217; US 2004175695 A1 Provisional US 2002-436591P 20021226, US 2003-736899 20031217; AU 2003293562 A1 AU 2003-293562 20031217; EP 1604183 A2 EP 2003-790511 20031217, WO 2003-US39938 20031217
FDT AU 2003293562 A1 Based on WO 2004059280; EP 1604183 A2 Based on WO 2004059280
PRAI US 2002-436591P 20021226; US 2003-736899 20031217

AB WO2004059280 A UPAB: 20040818
NOVELTY - Measuring different organisms in a sample comprises contacting the sample with an ***extraction*** reagent comprising ***nitrous*** ***acid*** , thus forming an assay composition, and measuring in the assay composition, markers of the organisms so as to measure the different organisms.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a kit for measuring different organism types in a sample comprising, in one or more containers:

(a) an acid;

(b) a nitrite salt;

(c) a surfactant;

(d) a first binding reagent that binds a first marker from a first of the different organism types or a first antibody that binds a first marker that is a cell wall-associated antigen from a Streptococcus bacterium or a group specific antigen from Streptococci Group A, B, F or G bacteria; and

(e) a second binding reagent that binds a second marker from a second of the different organism types or a second antibody that binds a second marker from a ***virus*** or influenza A; and

(f) a third antibody that binds a third marker from influenza B and a fourth antibody that binds a fourth marker from respiratory syncytial ***virus*** (RSV);

(2) a method for ***extracting*** one or more or two or more markers from a matrix;

(3) a method for measuring one or more or two or more markers;

(4) a composition comprising two or more markers and an oxidizing acid, one or more markers and an oxidizing acid, where at least one marker is a protein, peptide, toxin, nucleic acid, or lipid, or one or more markers and an oxidizing acid, where at least one marker is a viral or fungal marker;

(5) a kit for ***extracting*** two or more (or one or more) markers from a sample for use in one or more assays comprising, in one or more containers:

(a) an acid;

(b) a nitrite salt;

(c) a surfactant; and

(d) at least one ***extraction*** component selected from:

(i) a pH buffer/pH neutralizer;

(ii) a sampler;

(iii) preservatives;

(iv) stabilizing agents;

(v) ***extraction*** vessel;

(vi) bleach;

(vii) desiccants;

(viii) capture moiety; and

(ix) detection moiety, (where at least one of the one or more markers is a viral or fungal marker or a protein, nucleic acid or lipid marker); and

(6) a clinical method for ***extracting*** markers of pathogenic microorganisms responsible for a disease of the upper respiratory tract.

USE - The methods are useful for measuring microorganisms particularly for ***extracting*** markers of pathogenic microorganisms responsible for a disease of the upper respiratory tract such as pharingytis, sinusitis, pneumonia, bronchitis, flu and/or the common cold. The methods are also useful for diagnosing such diseases in patients. The kit is useful for immunoassay, immunochromatographic assay, and specific assay.

Dwg.0/0

L3 ANSWER 8 OF 17 IFIPAT COPYRIGHT 2006 IFI on STN
AN 10417400 IFIPAT;IFIUDB;IFICDB
TI THERAPEUTIC AND COSMETIC USES OF HEPARANASES; INDUCING WOUND HEALING AGENTS; ANTIINFLAMMATORY AGENTS; ANTITUMOR AGENTS
INF Feinstein; Elena, Rehovot, IL
Ilan; Neta, Rehovot, IL
Pecker; Iris, Rishon LeZion, IL
Vlodavsky; Israel, Mevaseret Zion, IL
Yacoby-Zeevi; Oron, Moshav Bizaron, IL
IN Feinstein Elena (IL); Ilan Neta (IL); Pecker Iris (IL); Vlodavsky Israel (IL); Yacoby-Zeevi Oron (IL)
PAF Unassigned

PA Unassigned Or Assigned To Individual (68000)
PPA Hadasit Medical Research Services and Development Co IL (Probable)
AG G.E. EHRLICH (1995) LTD. c/o ANTHONY CASTORINA, SUITE 207, 2001 JEFFERSON DAVIS HIGHWAY, ARLINGTON, VA, 22202, US
PI US 2003161823 A1 20030828
AI US 2003-341582 20030114
RLI US 1999-258892 19990301 CONTINUATION
US 2001-776874 20010206 CONTINUATION
WO 1998-US17954 19980831 CONTINUATION-IN-PART
WO 2001-IL830 20010905 CONTINUATION-IN-PART
US 2001-988113 20011119 CONTINUATION-IN-PART
FI US 2003161823 20030828
DT Utility; Patent Application - First Publication
FS CHEMICAL
APPLICATION
OS CA 139:193995
PARN This is a continuation-in-part of U.S. patent application No. 09/988,113, filed Feb. 6, 2001, which is a continuation of U.S. patent application No. 09/776,874, filed Feb. 6, 2001, which is a continuation of U.S. patent application No. 09/258,892, filed Mar. 1, 1999, which is a continuation-in-part of PCT/US98/17954, filed Aug. 31, 1998, which claims priority from U.S. patent application 09/109,386, filed Jul. 2, 1998, now abandoned, which is a continuation-in-part of U.S. patent application 08/ 922,170, filed Sep. 2, 1997, now, U.S. Pat. No. 5,968,822. This application is also a continuation-in-part of PCT/IL01/00830, filed Sep. 5, 2001, which claims the benefit of priority from U.S. patent application No. 09/727,479, filed Dec. 4, 2000, which claims the benefit of priority from U.S. Provisional Patent Application Nos. 60/231,551, filed Sep. 11, 2000, and 60/ 244,593, filed Nov. 1, 2000.
CLMN 84
GI 35 Figure(s).

FIG. 1 presents nucleotide sequence and deduced amino acid sequence of hpa cDNA. A single nucleotide difference at position 799 (A to T) between the EST (Expressed Sequence Tag) and the PCR amplified cDNA (reverse transcribed RNA) and the resulting amino acid substitution (Tyr to Phe) are indicated above and below the substituted unit, respectively. Cysteine residues and the poly adenylation consensus sequence are underlined. The asterisk denotes the stop codon TGA.

FIG. 2 demonstrates degradation of soluble sulfate labeled HSPG substrate by lysates of High Five cells infected with pFhpa2 ***virus*** . Lysates of High Five cells that were infected with pFhpa2 ***virus*** (*) or control pF2 ***virus*** (*) were incubated (18 h, 37 degrees C.) with sulfate labeled ECM-derived soluble HSPG (peak I). The incubation medium was then subjected to gel filtration on Sepharose 6B. Low molecular weight HS degradation fragments (peak II) were produced only during incubation with the pFhpa2 infected cells, but there was no degradation of the HSPG substrate (open-diamond) by lysates of pF2 infected cells.

FIGS. 3a-b demonstrate degradation of soluble sulfate labeled HSPG substrate by the culture medium of pFhpa2 and pFhpa4 infected cells. Culture media of High Five cells infected with pFhpa2 (3a) or pFhpa4 (3b) ***viruses*** (*), or with control ***viruses*** (*) were incubated (18 h, 37 degrees C.) with sulfate labeled ECM-derived soluble HSPG (peak I, open-diamond). The incubation media were then subjected to gel filtration on Sepharose 6B. Low molecular weight HS degradation fragments (peak II) were produced only during incubation with the hpa gene containing ***viruses*** . There was no degradation of the HSPG substrate by the culture medium of cells infected with control ***viruses*** .

FIG. 4 presents size fractionation of heparanase activity expressed by pFhpa2 infected cells. Culture medium of pFhpa2 infected High Five cells was applied onto a 50 kDa cut-off membrane. Heparanase activity (conversion of the peak I substrate, (open-diamond) into peak II HS degradation fragments) was found in the high (greater-than 50 kDa) (*), but not low (less-than 50 kDa) (o) molecular weight compartment.

FIGS. 5a-b demonstrate the effect of heparin on heparanase activity expressed by pFhpa2 and pFhpa4 infected High Five cells. Culture media of pFhpa2 (5a) and pFhpa4 (5b) infected High Five cells were incubated (18 h, 37 degrees C.) with sulfate labeled ECM-derived soluble HSPG (peak I, open-diamond) in the absence (*) or presence (Delta) of 10 mu g/ml heparin. Production of low molecular weight HS degradation fragments was completely abolished in the presence of heparin, a potent inhibitor of

heparanase activity (6, 7).

FIGS. 6a-b demonstrate degradation of sulfate labeled intact ECM by ***virus*** infected High Five and Sf21 cells. High Five (6a) and Sf21 (6b) cells were plated on sulfate labeled ECM and infected (48 h, 28 degrees C.) with pFhpa4 (*) or control pF1 (*) ***viruses*** . Control non-infected Sf21 cells (R) were plated on the labeled ECM as well. The pH of the cultured medium was adjusted to 6.0-6.2 followed by 24 h incubation at 37 degrees C. Sulfate labeled material released into the incubation medium was analyzed by gel filtration on Sepharose 6B. HS degradation fragments were produced only by cells infected with the hpa containing ***virus*** .

FIG. 7a-b demonstrate degradation of sulfate labeled intact ECM by ***virus*** infected cells. High Five (7a) and Sf21 (7b) cells were plated on sulfate labeled ECM and infected (48 h, 28 degrees C.) with pFhpa4 (*) or control pFl (*) ***viruses*** . Control non-infected Sf21 cells (r) were plate on labeled ECM as well. The pH of the cultured medium was adjusted to 6.0-6.2, followed by 48 h incubation at 28 degrees C. Sulfate labeled degradation fragments released into the incubation medium was analyzed by gel filtration on Sepharose 6B. HS degradation fragments were produced only by cells infected with the hpa containing ***virus*** .

FIGS. 8a-b demonstrate degradation of sulfate labeled intact ECM by the culture medium of pFhpa4 infected cells. Culture media of High Five (8a) and Sf21 (8b) cells that were infected with pFhpa4 (*) or control pF1 (E) ***viruses*** were incubated (48 h, 37 degrees C., pH 6.0) with intact sulfate labeled ECM. The ECM was also incubated with the culture medium of control noninfected Sf21 cells (r). Sulfate labeled material released into the reaction mixture was subjected to gel filtration analysis. Heparanase activity was detected only in the culture medium of pFhpa4 infected cells.

FIGS. 9a-b demonstrate the effect of heparin on heparanase activity in the culture medium of pFhpa4 infected cells. Sulfate labeled ECM was incubated (24 h, 37 degrees C., pH 6.0) with culture medium of pFhpa4 infected High Five (9a) and Sf21 (9b) cells in the absence (*) or presence (V) of 10 mu g/ml heparin. Sulfate labeled material released into the incubation medium was subjected to gel filtration on Sepharose 6B. Heparanase activity (production of peak II HS degradation fragments) was completely inhibited in the presence of heparin.

FIGS. 10a-b demonstrate purification of recombinant heparanase on heparin-Sepharose. Culture medium of Sf21 cells infected with pFhpa4 ***virus*** was subjected to heparin-Sepharose chromatography. Elution of fractions was performed with 0.35-2 M NaCl gradient (open-diamond). Heparanase activity in the eluted fractions is demonstrated in FIG. 10a (*). Fractions 1528 were subjected to 15% SDS-polyacrylamide gel electrophoresis followed by silver nitrate staining. A correlation is demonstrated between a major protein band (MW*63,000) in fractions 19-24 and heparanase activity.

FIGS. 11a-b demonstrate purification of recombinant heparanase on a Superdex 75 gel filtration column. Active fractions eluted from heparin-Sepharose (FIG. 10a) were pooled, concentrated and applied onto Superdex 75 FPLC column. Fractions were collected and aliquots of each fraction were tested for heparanase activity (c, FIG. 11a) and analyzed by SDS-polyacrylamide gel electrophoresis followed by silver nitrate staining (FIG. 11b). A correlation is seen between the appearance of a major protein band (MW*63,000) in fractions 4-7 and heparanase activity.

FIGS. 12a-e demonstrate expression of the hpa gene by RT-PCR with total RNA from human embryonal tissues (12a), human extraembryonal tissues (12b) and cell lines from different origins (12c-e). RT-PCR products using hpa specific primers (I), primers for GAPDH housekeeping gene (II), and control reactions without reverse transcriptase demonstrating absence of genomic DNA or other contamination in RNA samples (III). M-DNA molecular weight marker VI (Boehringer Mannheim). For 12a: lane 1-neutrophil cells (adult), lane 2-muscle, lane 3-thymus, lane 4-heart, lane 5-adrenal. For 12b: lane 1-kidney, lane 2placenta (8 weeks), lane 3-placenta (11 weeks), lanes 4-7-mole (complete hydatidiform mole), lane 8-cytotrophoblast cells (freshly isolated), lane 9-cytotrophoblast cells (1.5 h in vitro), lane 10-cytotrophoblast cells (6 h in vitro), lane 11cytotrophoblast cells (18 h in vitro), lane 12-cytotrophoblast cells (48 h in vitro). For 12c: lane 1-JAR bladder cell line, lane 2-NCITT testicular tumor cell line, lane 3-SW-480 human hepatoma cell line, lane 4-HTR (cytotrophoblasts transformed by SV40), lane 5-HPTLP-I hepatocellular carcinoma cell line, lane 6-EJ-28 bladder carcinoma cell

line. For 12d: lane 1-SK-hep-1 human hepatoma cell line, lane 2-DAMI human megakaryocytic cell line, lane 3-DAMI cell line+PMA, lane 4-CHRF cell line+PMA, lane 5-CHRF cell line. For 12e: lane 1-ABAE bovine aortic endothelial cells, lane 2-1063 human ovarian cell line, lane 3human breast carcinoma MDA435 cell line, lane 4-human breast carcinoma MDA231 cell line.

FIG. 13 presents a comparison between nucleotide sequences of the human hpa and a mouse EST cDNA fragment (SEQ ID NO: 12) which is 80% homologous to the 3' end (starting at nucleotide 1066 of SEQ ID NO:9) of the human hpa. The aligned termination codons are underlined.

FIG. 14 demonstrates the chromosomal localization of the hpa gene. PCR products of DNA derived from somatic cell hybrids and of genomic DNA of hamster, mouse and human of were separated on 0.7% agarose gel following amplification with hpa specific primers. Lane 1-Lambda DNA digested with BstEII, lane 2-no DNA control, lanes 3-29, PCR amplification products. Lanes 3-5human, mouse and hamster genomic DNA, respectively. Lanes 6-29, human monochromosomal somatic cell hybrids representing chromosomes 1-22 and X and Y, respectively. Lane 30-Lambda DNA digested with BstEII. An amplification product of approximately 2.8 Kb is observed only in lanes 5 and 9, representing human genomic DNA and DNA derived from cell hybrid carrying human chromosome 4, respectively. These results demonstrate that the hpa gene is localized in human chromosome 4.

FIG. 15 demonstrates the genomic exon-intron structure of the human hpa locus (top) and the relative positions of the lambda clones used as sequencing templates to sequence the locus (below). The vertical rectangles represent exons (E) and the horizontal lines therebetween represent introns (I), upstream (U) and downstream (D) regions. Continuous lines represent DNA fragments, which were used for sequence analysis. The discontinuous line in lambda 6 represent a region, which overlaps with lambda 8 and hence was not analyzed. The plasmid contains a PCR product, which bridges the gap between L3 and L6.

FIG. 16 presents the nucleotide sequence of the genomic region of the hpa gene. Exon sequences appear in upper case and intron sequences in lower case. The deduced amino acid sequence of the exons is printed below the nucleotide sequence. Two predicted transcription start sites are shown in bold.

FIG. 17 presents an alignment of the amino acid sequences of human heparanase, mouse and partial sequences of rat homologues. The human and the mouse sequences were determined by sequence analysis of the isolated cDNAs. The rat sequence is derived from two different EST clones, which represent two different regions (5' and 3') of the rat hpa cDNA. The human sequence and the amino acids in the mouse and rat homologues, which are identical to the human sequence, appear in bold.

FIG. 18 presents a heparanase Zoo blot. Ten micrograms of genomic DNA from various sources were digested with EcoRI and separated on 0.7% agarose-TBE gel. Following electrophoresis, the was gel treated with HCl and than with NaOH and the DNA fragments were downward transferred to a nylon membrane (Hybond N+, Amersham) with 0.4 N NaOH. The membrane was hybridized with a 1.6 Kb DNA probe that contained the entire hpa cDNA. Lane order: H-Human; M-Mouse; Rt-Rat; P-Pig; Cw-Cow; Hr-Horse; SSheep; Rb-Rabbit; D-Dog; Ch-Chicken; F-Fish. Size markers (Lambda BsteII) are shown on the left

FIG. 19 demonstrates the secondary structure prediction for heparanase performed using the PHD server-Profile network Prediction Heidelberg. H-helix, E-extended (beta strand), The glutamic acid predicted as the proton donor is marked by asterisk and the possible nucleophiles are underlined.

FIGS. 20a-b demonstrate the expression of heparanase by human endothelium. 20a-RT-PCR. Total RNA isolated from ECGFstimulated proliferating human umbilical vein (HUVEC, lane 1) and bone marrow (TrHBMEC, lane 2) derived EC was analyzed by RTPCR for expression of the heparanase mRNA, using human specific hpa primers amplifying a 564 bp cDNA (Vlodavsky, I. et al. Mammalian heparanase: gene cloning, expression and function in tumor progression and metastasis. Nat Med 5, 793-802 (1999)) fragment. Lane 3, DNA molecular weight markers. 20bImmunohistochemistry. Immunostaining of tissue specimens was performed as described in the Examples section that follows. Positive staining is reddish-brown. Preferential staining of the heparanase protein is seen in the endothelium of capillaries and small sprouting vessels (arrows, left & right panels) as compared to little or no staining of endothelial cells in mature quiescent blood vessels (concave arrows, left & middle panels). Enhanced expression of the heparanase protein is seen in the neoplastic colonic epithelium. Original

magnification is 200 x (left and right panels) and 100 x (middle panel).

FIGS. 21a-c demonstrate release of ECM-bound bFGF by recombinant heparanase, and bFGF accessory activity of HS degradation fragments released from EC vs. ECM. 21a-b-Release of ECM-bound bFGF. 21a-ECM-coated wells of four-well plates were incubated (3 hours, 24 degrees C.) with 125I-bFGF as described in the Examples section that follows. The ECM was washed 3 times and incubated (3 hours, 37 degrees C.) with increasing concentrations of recombinant heparanase. Released radioactivity is expressed as percent of the total ECM-bound 125I-bFGF. About 10% of the ECM-bound 125I-bFGF was released in the absence of added heparanase. Each data point is the mean +SD of triplicate wells. Where error bars cannot be seen, SD is smaller than the symbol. 21a (inset)-Release of sulfate labeled HS degradation fragments. Metabolically sulfate labeled ECM was incubated (3 hours, 37 degrees C., pH 6.0) with 0.2 mu g/ml recombinant heparanase. Sulfate labeled material released into the incubation medium was analyzed by gel filtration on Sepharose 6B. Labeled fragments eluted in fractions 15-35 (peak II) were 5-6 fold smaller than intact HS side chains and were susceptible to deamination by ***nitrous*** ***acid*** (Vlodavsky, I. et al. Mammalian heparanase: gene cloning, expression and function in tumor progression and metastasis. Nat Med 5, 793-802 (1999)). 21b-Release of endogenous ECM-resident bFGF by heparanase. Recombinant heparanase (0.5 mu g/ml) was incubated (4 hours, 37 degrees C.) with ECM coated 35-mm dishes in 1 ml heparanase reaction mixture. Aliquots of the incubation media were taken for quantitation of bFGF by ELISA as described in the Examples section that follows. Each data point is the mean +-S.D. of triplicate determinations. 21c-Stimulation of bFGF induced DNA synthesis in BaF3 lymphoid cells by HS degradation fragments. Confluent bovine aortic EC cultured in 35-mm plates and their underlying ECM (as described in Gospodarowicz D. Moran J Braun D and Birdwell C 1976 Clonal growth of bovine vascular endothelial cells: fibroblast growth factor as a survival agent. Proc. Natl. Acad. Sci. 73: 4120-4124) were incubated (4 hours, 37 degrees C., pH 6.5) with 0.1 mu g/ml recombinant heparanase. Aliquots (5-200 mu l) of the incubation media were then added to BaF3 cells seeded into 96 well plates in the presence of 5 ng/ml bFGF. 3H-thymidine (1 mu Ci/well) was added 48 hours after seeding and 6 hours later the cells were harvested and measured for 3H-thymidine incorporation. Each data point represents the mean +-S.D. of six culture wells. 21c (Inset)Release of sulfate labeled material from EC (open circles) vs. ECM (closed circles). In control plates, both the EC and ECM were first metabolically labeled with Na2(35S)O4. Sulfate labeled material released by heparanase (0.2 mu g/ml, 4 hours, 37 degrees C.) from EC and ECM was subjected to gel filtration.

FIGS. 22a-c demonstrate angiogenic response induced by Matrigel embedded with hpa vs. mock transfected Eb lymphoma cells. BALB/ c mice (n=5) were injected subcutaneously with 0.4 ml cold Matrigel premixed with 2 x 106 hpa- or mock-transfected Eb lymphoma cells. After 7 days, the mice were sacrified, and the Matrigel plugs were removed and photographed. Angiogenic response was then quantitated by measurement of the hemoglobin content as described in the Examples section that follows. 22aRepresentative Matrigel plugs containing hpa transfected (left) and mock transfected (right) Eb cells photographed in situ, prior to their removal out of their subcutaneous location in the mice. 22b-Matrigel plugs containing heparanase producing (bottom) vs. control mock transfected (top) Eb cells. Shown are isolated Matrigel plugs removed from 10 different mice. 22cHemoglobin content of Matrigel plugs (shown in FIG. 22b) containing hpa transfected (dark bar) vs. control mock transfected (empty bar) Eb cells. Represented is the mean +-SD (n=5, p=0.0089; unpaired t test).

FIGS. 23a-b demonstrate that topical administration of active heparanase accelerate wound healing. 23a-Full-thickness wounds were created with a circular 8 mm punch at the back of the mouse skin. Wound areas were calculated after 7 days in control (1) or active heparanase-treated (2) mice and are shown as total area (23a) and percent (23b). Note the enhancement of wound healing upon exogenous application of heparanase. Data are statistically significant (P values equals 0.0023).

FIGS. 24a-d demonstrate an increase in granulation tissue cellularity upon heparanase treatment. Full-thickness wounds were created as described for FIGS. 24a-b. Wounds were left untreated (24a-b) or treated with heparanase for 7 days (24c-d). Wounds, including the underlying granulation tissue were formalin-fixed, paraffin-embedded and 5 micron sections were stained with hematoxilin-eosin. Note the increase in the granulation tissue cellularity upon heparanase treatment. Original

magnifications: 24a and 24c x 170; 24b and 24d x 340.

FIGS. 25a-f demonstrate that heparanase treatment induces cellular proliferation and granulation tissue vascularization. Five micron sections from non-treated (25a, c and d) and heparanase-treated (25b, e and f) granulation tissues were stained for PCNA (25a-b and 25d-e) and for PECAM-1 (25c, f). Note the increase in PCNA-positive cells and PECAM-1 positive blood vessels structures upon heparanase treatment. Original magnifications: 25a-c x 170, 25d-f x 340.

FIGS. 26a-f demonstrates that heparanase expression is restricted to differentiated keratinocytes in mouse skin tissue. Five micron skin tissue sections were stained for PCNA (26a, d) and heparanase (26b-c, e). Negative control (no primary antibody) is shown in 26f. Note intense PCNA staining at the basal epidennal cell layer (26a, d) while heparanase mainly stain the outer most, keratinocytes, cell layer (26b, e) and the cells composing the hair follicle (26c). In the latter case, nuclear staining is observed.

FIGS. 27a-d demonstrate expression of heparanase in human skin. 27a-cultures of HaCat keratinocytes cell line immunostained with anti-heparanase monoclonal antibody (HP-92). 27bheparanase activity in intact cells and in ***extracts*** of HaCat cells, in an ECM-assay. 27c and d-immuno-staining of normal skin tissue with HP-92.

FIG. 28 demonstrates stimulation of angiogenesis by heparanase in rat eye model. The central cornea of rats' eyes was scraped with a surgical knife. The right eye of each rat was then treated with heparanase, 50 mu l drop (1 mg/ml) of purified recombinant human P50 heparanase, three times a day. The left eye served as a control and was treated with Lyeteers. Vascularization and epithelialization were evaluated following closure of the corneal lesion. Heparanase treated eyes exhibited vascularization of the cornea, as well as increased vascularization in the iris. Normal, minor vascularization of the iris and non vascular appearance of the cornea were observed in the controls

FIG. 29 demonstrates cornea sections of heparanase treated eye as compared to control, Lyeteers treated eyes. Control eyes demonstrate healing of the epithelia which is accompanied by a normal organized structure of the cornea. Heparanase treatment resulted in growth of blood vessels into the cornea (arrows), followed by a massive infiltration of lymphocytes. Vascularization associated inflammatory reaction interfered with corneal healing, as demonstrated by a disorganized structure of the cornea.

FIGS. 30a-e demonstrate that skin tissue morphology is impaired under diabetic conditions. Skin sections from normal (30a, 30d) and streptozotocin-induced diabetic (b, e) rats were hematoxilin-eosin stained (30a, 30b) or immunostained with antiheparanase antibodies (30d, 30e). Measurements from 10 control or diabetic different rats are shown in (30c). Note a dramatic decrease in the skin tissue thickness and reduced heparanase expression under diabetic conditions.

FIGS. 31a-f demonstrate heparanase expression in the wound granulation tissue. Full-thickness wounds were generated by 8 mm punch at the back of rat skin. Seven days later the wounds, including the newly formed granulation tissue, were harvested, formalin-fixed and paraffin-embedded. Five microns sections were stained for heparanase (31a-c), or double stained for heparanase (red) and SMA (green)(31d-f). Note heparanase expression in the granulation tissue (31a) and at the lumenfacing areas of endothelial cells lining blood vessels (31e, 31f). Original magnifications: a x 4, b x 10,c-f x 40.

FIG. 32 demonstrates that heparanase accelerates wound healing in streptozotocin-induced rat diabetic. Four 8 mm fullthickness punches were created at the back of normal, nondiabetic (Nor), or diabetic rats. Wounds were treated with saline (Nor, Con), heparanase (Hep, 1 mu g/wound) or PDGF (0.5 mu g/wound) immediately following wounding, four hours later, and three additional times during the following day, at 4 hours intervals. Seven days after wounding, wounds were harvested, fixed and wound closure was evaluated under low power magnification of hematoxilin-eosin stained sections. Three animals were included in each group to yield 12 wounds for each treatment. Note improved wound healing upon heparanase treatment, similar to PDGF effect.

FIGS. 33a-b demonstrate that heparanase accelerates wound healing under ischemic conditions. FIG. 33a is a schematic representation of the flap/punch ischemic wound model. Two longitudinal incisions, each 6 cm in length, were connected at the caudal end with a third, 3 cm, incision across the midline. The flap was elevated to the base of the camial pedicle, replaced in its bed and secured with sutures. Two 8 mm punches were generated in the flap 3 cm from the carnial end. FIG. 33bWounds were

treated with saline (Con), active heparanase (p45, 1 mu g/wound), the heparanase precursor (p60, 5 mu g/wound) and PDGF (0.5 mu g/wound) immediately after wounding, 4 hours later and three more times, 4 hours apart, the next day (a total of 5 application, each at a volume of 50 mu l). Longitude incisions were treated once just prior to clipping. Wounds closure was evaluated 10 days following wounding by histological examination. P45 as well as p60 heparanases significantly improved wound closure (p values are 0.03 and 0.016 for p45 and p60, respectively). Five rats were included in each group, and two wounds were created at each flap to yield a total of 10 wounds.

FIG. 34 demonstrates that heparanase induces reepithalialization of incisional wounds. Typical histological examination of control (left) and heparanase (p45)-treated incisional wounds from the flap described in FIGS. 33a-b is shown. Measurements of 10 incisions from control and heparanase treated incisions are shown graphically. Note a robust increase in the epithelial layer thickness upon heparanase treatment.

FIG. 35 demonstrates that heparanase treatment induces the recruitment of pericytes into blood vessels. Untreated (Con) and heparanase-treated (Hep) wound sections from the ischemic model were immunostained with anti-SMA antibodies. Representative photomicrographs are shown on the left and graphical evaluation of 10 different wounds, and at least 3 different fields in each wound, is shown on the right. Note the dramatic recruitment of SMA-positive pericytes into blood vessels upon heparanase treatment. ! OF 17 IFIPAT COPYRIGHT 2006 IFI on STN

AB Methods and compositions for inducing and/or accelerating wound healing and/or angiogenesis via the catalytic activity of heparanase are disclosed.

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DUPLICATE 2
AN 1992:394498 BIOSIS
DN PREV199294066673; BA94:66673
TI ARTIFICIAL INDUCTION AND EVALUATION OF A MILD ISOLATE OF TOMATO SPOTTED WILT VIRUS.
AU WANG M [Reprint author]; GONSALVES D
CS 3190 MAILE WAY, ST JOHN, DEP PLANT PATHOL, UNIV HAWAII, HONOLULU, HAWAII 96822, USA
SO Journal of Phytopathology (Berlin), (1992) Vol. 135, No. 3, pp. 233-244.
CODEN: JPHYEB. ISSN: 0931-1785.
DT Article
FS BA
LA ENGLISH
ED Entered STN: 24 Aug 1992
Last Updated on STN: 24 Aug 1992
AB A severe isolate (BL) of tomato spotted wilt ***virus*** (TSWV) that originated from Hawaii [USA] was treated with ***nitrous*** ***acid*** in an effort to obtain mild mutants. The standardized procedure used in mutation experiments was: ***extracting*** infected Gomphrena globosa. L. leaf tissue in 0.01 M Na2SO3, 0.125 M sodium acetate and 0.4 M sodium nitrite at pH 5.5 and incubating the ***extract*** for 20 min at room temperature. The ***extract*** was inoculated to tobacco (Nicotiana tabacum L. cv. Havana 423) and local lesions were subsequently transferred to lettuce (Lactuca sativa L. cv. Minetto). One isolate (R27G) that incited mild symptoms in lettuce was obtained out of 868 local-lesion-transfers. Under greenhouse conditions, the isolate induced mild symptoms on tomato (Lycopersicon esculentum Mill.) but was severe on peppers (Capsicum annuum L.). The effect of the R27G isolate on growth of potted tomatoes kept outdoors was variable. In one trial, only 15% of the fruit had symptoms versus 67% in another trial. R27G fully protected Datura stramonium L. plants that were challenge inoculated with the severe parent BL isolate. Less effective cross protection was observed against a severe isolate from Oklahoma [USA].

L3 ANSWER 10 OF 17 DISSABS COPYRIGHT (C) 2006 ProQuest Information and Learning Company; All Rights Reserved on STN
AN 90:23236 DISSABS Order Number: AAR9106337
TI TOMATO SPOTTED WILT VIRUS: SEROLOGY, CROSS PROTECTION, MOLECULAR CLONING AND RELATEDNESS WITH BUNYAVIRIDAE (VIRUS)
AU WANG, MIN [PH.D.]
CS CORNELL UNIVERSITY (0058)
SO Dissertation Abstracts International, (1990) Vol. 51, No. 9B, p. 4138.
Order No.: AAR9106337. 134 pages.

DT Dissertation
FS DAI
LA English
ED Entered STN: 19921118
Last Updated on STN: 19921118
AB A mild isolate (R27G) of tomato spotted wilt ***virus*** (TSWV) was selected after ***nitrous*** ***acid*** treatment of a severe isolate (BL). The intention was to use a mild isolate in cross protection of lettuce and tomatoes against TSWV infection. Under greenhouse conditions, R27G-infected lettuce and tomatoes showed mild symptoms compared with those of the severe isolate, although the results of outdoor experiments varied. The mild isolate fully protected Datura stramonium against the parent BL isolate. Double sandwich direct enzyme-linked immunosorbent assay (ELISA) with antibodies to whole virions is recommended for ***virus*** detection since the assay detected all 30 collected TSWV isolates. Antibodies to the 26 kDa nucleoprotein and 78 kDa membrane protein of TSWV detected 19 of the 30 isolates in various ELISA tests, while the other 11 gave inconsistent results. Although TSWV has been reported to be similar to ***viruses*** in the family of Bunyaviridae, no serological relatedness was observed between TSWV and ***viruses*** in the Phlebovirus genus of Bunyaviridae. ***Viruses*** in Bunyaviridae replicate in mosquito Toxorhynchites amboinensis. High antigen levels of TSWV were detected by ELISA tests of injected mosquitoes with purified TSWV during the first week, and were at detectable levels up to 5 weeks later. Infectivity was not recovered from these mosquito ***extracts*** and detections of TSWV RNA by cDNA hybridizations were not consistent. TSWV RNAs were cloned. Six clones reacted specifically to S RNA of TSWV in Northern blots. Three hybridized to M RNA. No hybridization to healthy D. stramonium was observed for these clones in Northern and dot blot hybridizations. Heterogeneity of S RNA within BL isolate is suspected since some S RNA clones of the BL isolate reacted positively while others reacted negatively with a synthetic oligo nucleotide probe made according to the published sequence of the nucleoprotein gene of a TSWV isolate from the Netherlands. No homology was found between partial sequences of two TSWV S RNA clones and published TSWV S RNA sequence.

L3 ANSWER 11 OF 17 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 3
AN 1990:204697 BIOSIS
DN PREV199089111368; BA89:111368
TI COMPARATIVE STUDIES ON IN-VITRO TRANSLATION OF A SEVERE STRAIN AND A MILD STRAIN OF PAPAYA RINGSPOT VIRUS.
AU YEH S-D [Reprint author]; BIH F-Y
CS DEP PLANT PATHOL, NATL CHUNG HSING UNIV, TAICHUNG
SO Plant Protection Bulletin (Taichung), (1989) Vol. 31, No. 3, pp. 276-289.
CODEN: PLPBBH. ISSN: 0577-750X.
DT Article
FS BA
LA ENGLISH
ED Entered STN: 24 Apr 1990
Last Updated on STN: 24 Apr 1990
AB Papaya ringspot ***virus*** (PRV) strain HA is a severe strain of the ***virus*** . In vitro translation of PRV HA RNA in the wheat germ system showed that proteins of 64,000 daltons (64K), 46K, and 24K were three major products; also, a 112K protein was detected under certain conditions. The products of 112K, 100K, 84K, 76K, and 51K reacted with with the antiserum to amorphous inclusion protein (AIP) of the ***virus*** . However, the 64K protein was not related to AIP, coat protein (CP), or cylindrical inclusion protein (CIP). In addition, proteins of 46K, 24K and 14K reacted with the CP antiserum. Products corresponding to authentic CIP and CP were not detected. PRV HA 5-1 is a ***nitrous*** - ***acid*** induced mild mutant for control of the papaya ringspot disease by cross protection. Comparison of products translated in wheat germ ***extracts*** revealed that corresponding to the 20K product synthesized by HA RNA a 19K product was generated by HA 5-1 RNA. Also, the intensity of the 23K protein directed by HA 5-1 RNA was much high stronger than that of HA RNA. Another difference was that for the 50K and 52K products synthesized by HA RNA only one corresponding product of 51K was generated by Ha 5-1 RNA. Analyses of translation products synthesized in the rabbit reticulocyte lysate revealed that in the positions of 22K and 20K proteins generated by HA RNA, two

corresponding products of 21K and 19K were synthesized HA 5-1 RNA. Other differences in 43K and 34K products were also noticed but not consistent in different experiments. Results of in vitro translation directed by PRV HA and HA 5-1 RNAs in wheat germ ***extracts*** and rabbit reticulocyte lysates indicated that the general pattern of their translation products were the same but minor differences did exist. The difference in high-molecular-weight proteins synthesized by HA RNA and HA 5-1RNAs in two cell-free systems was not detected. The consistent differences between translation patterns of HA and HA 5-1 in the two cell-free systems were that a 20K product was directed by HA RNA but in the corresponding position 19K product was synthesized by HA 5-1 RNA. When translation products were assayed by immunoprecipitation, all different products between the severe and the mild strains did not react with antisera to PRV CP, AIP or CIP.

L3 ANSWER 12 OF 17 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 4
AN 1983:288726 BIOSIS
DN PREV198376046218; BA76:46218
TI USE OF TEMPERATURE SENSITIVE MUTANTS TO STUDY THE MORPHOGENESIS OF POLIOVIRUS.
AU DRESCHER-LINCOLN C K [Reprint author]; PUTNAK J R; PHILLIPS B A
CS DEP MICROBIOL, SCH MED, UNIV PITTSBURGH, PITTSBURGH, PA 15261, USA
SO Virology, (1983) Vol. 126, No. 1, pp. 301-316.
CODEN: VIRLAX. ISSN: 0042-6822.
DT Article
FS BA
LA ENGLISH
AB Three temperature-sensitive (ts) mutants of poliovirus (type 1 Mahoney) were isolated after ***nitrous*** ***acid*** treatment and characterized as phenotypically RNA+. When human cervical carcinoma HeLa cells were infected at 37.degree. C with 2 of the 3 RNA+ ts mutants (ts109 and ts739), reduced levels of 14 S particles were synthesized. One RNA+ mutant (ts520) synthesized significant amounts of viral 14 S particle subunits. All of the mutants synthesized reduced amounts of procapsids and virions at 37.degree. C. At 39.5.degree. C, with all 3 ts mutants, the production of all ***virus*** -related particles in infected cells was markedly suppressed. Isoelectric focusing of the viral-related particles produced at 37.degree. C by the ts mutants and electrophoretic analysis of their structural polypeptides revealed the following: ts739 synthesized an altered VPO polypeptide and produced 14 S particles with an altered isoelectric point; ts109 produced 14 S particles with a normal isoelectric (pI) but containing what appeared to be an altered VPI; ts520 produced normal 14 S particles as demonstrated by their pI, the electrophoretic behavior of their constituent structural polypeptides in sodium dodecyl sulfate-polyacrylamide gel electrophoresis, their abiity to self-assemble and their ability to form procapsid-like structures when incubated in ***extracts*** from wild-type (wt) ***virus*** -infected cells. Few, if any, procapsids were contained in ts520-infected cells and ***extracts*** made therefrom were unable to assemble ts520 or wt 14 S particles into detectable amounts of pI 6.8 empty capsids. These and other findings are consistent with ts739 (and probably ts109) possessing an altered structural protein and ts520 being mutant in its morphopoietic factor.

L3 ANSWER 13 OF 17 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
AN 1983-00197 BIOTECHDS
TI Isoelectric focusing and some other properties of a ***virus*** inhibitor purified from Phytolacca americana;
a plant virucide protein acting against tobacco-mosaic ***virus***
AU Kamijo H; Taniguchi T
LO Plant Pathology Laboratory, Faculty of Agriculture, Nagoya University, Chikusa-Ku, Nagoya, Japan.
SO Phytopathol.Z.; (1982) 104, 4, 316-24
CODEN: PHYZA3
DT Journal
LA English
AB The ordinary strain of tobacco-mosaic ***virus*** (TMV-OM) was propagated in leaves of Nicotiana tabacum. The frozen tissues infected with TMV-OM were homogenized with water and purified as a source of ***virus*** . Leaves of Phytolacca americana were homogenized and inhibitory activity was precipitated with ammonium sulfate. The

precipitated fraction was subjected to chromatography on Sephadex G-50 and DEAE-cellulose. French bean leaves were inoculated with a mixture of the P. americana ***extract*** , and TMV solution. The leaves contained very little TMV after 2 days incubation compared with control leaves. The isoelectric point of the purified inhibitor was estimated by CM-Sephadex column chromatography to be about 7.4. Of the many bands detected by isoelectric focusing, only one (pH 10.22) had strong inhibitor activity. It seems that some groups on the inhibitor molecule play an important role in inhibition since treatment with performic acid or ***nitrous*** ***acid*** resulted in 50-60% reduction of activity. (13 ref)

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AN 78186706 EMBASE
DN 1978186706
TI Biology of simian virus 40 (SV40) transplantation antigen (TrAg). II. Isolation and characterization of additional temperature sensitive mutants of SV40.
AU Tevethia M.J.; Ripper L.W.
CS Dept. Pathol., Tufts Univ. Sch. Med., Boston, Mass. 02111, United States
SO Virology, (1977) Vol. 81, No. 2, pp. 192-211. .
CODEN: VIRLAX
CY United States
DT Journal
FS 047 Virology
016 Cancer
005 General Pathology and Pathological Anatomy
LA English
AB Fourteen independent temperature sensitive mutants of simian ***virus*** (SV40) were isolated following ***nitrous*** ***acid*** or hydroxylamine mutagenesis. Three mutants were assigned to the A group and 7 to the BC group on the basis of standard qualitative and quantitative complementation assays. Three other mutants did not complement mutants of any complementation group well under standard conditions nor was delayed complementation observed in quantitative assays. However, these mutants were shown to complement members of the A and BC complementation groups but not members of the D group when the qualitative complementation test was modified by allowing the parental virions to uncoat at permissive temperature prior to incubation at 41.degree.. The assignment of these mutants to the D group was substantiated by demonstrating the wild type infectivity of DNA ***extracted*** from cells infected at 33.degree. for growth at 41.degree.. Thirteen of the mutants were tested for the production of tumor (T), capsid (C), virion (V), and major coat protein (VP1) antigens at permissive and nonpermissive temperature by immunofluorescence assays along with mutants which have been described previously by others for comparison. The temperature sensitive (ts) mutants isolated in this study produced fully immunoreactive T antigen at both temperatures. None of the tsA mutants produced C, VP1, or V antigens at elevated temperature. The BC mutants isolated in this study all produced T antigen at 41.degree.. These late mutants demonstrated 2 patterns of expression of virion antigens. One group synthesized C, V, and VP1 at 41.degree. and were indistinguishable from wild type on the basis of antigenic phenotype. A second group showed cytoplasmic and nucleolar fluorescence for C and VP1 antigens at the nonpermissive temperature similar to that observed for tsBC11 previously. Mutants in this group did not produce V antigen at high temperature.

L3 ANSWER 15 OF 17 NTIS COPYRIGHT 2006 NTIS on STN
AN 1966(31):05535 NTIS Order Number: AD-637 411/XAB
TI Inactivation of Two Arboviruses and Their Associated Infectious Nucleic Acids.
Reprint: Inactivation of Two Arboviruses and Their Associated Infectious Nucleic Acids.
AU Mika, L. A.; Officer, J. E.; Brown, A.
CS Army Biological Labs Frederick M (036550)
NR AD-637 411/XAB
2p; 12 Jun 1963
DT Report
CY United States
LA English
AV Published in Journal of Infectious Diseases v113 p195-203 Nov-Dec 1963.

NTIS Prices: Not available NTIS
OS GRA&I6619
AB The inactivation of 2 distinct but related arboviruses (Eastern and Venezuelan equine encephalitis) by heat (50 C), ***nitrous*** ***acid*** (HNO2), and ultraviolet light was studied in relation to the infectious ribonucleic acid (RNA). The 2 ***viruses*** could be distinguished by their heat inactivation curves. Although the curves for both ***viruses*** were approximately biphasic, their phases were reversed. The heat inactivation rates of recoverable RNA (from the heated ***virus*** particle) and of ***extracted*** RNA (from unheated ***virus***) were less than those for the ***virus*** . The results suggested that heat acts first on the surface (lipoprotein) component and then on the nucleic acid. The kinetics of inactivation of the 2 ***viruses*** and their RNA's by HNO2 suggested that inactivation of both surface protein and necleic acid began simultaneously but that the latter inactivation was slower. The respective ***viruses*** and their recoverable RNA could be distinguished by their rates of inactivation. The results with ultraviolet irradiation agreed with the concept of primary damage to the nucleic acid.

L3 ANSWER 16 OF 17 NIOSHTIC on STN
AN 1997:996 NIOSHTIC
DN NIOSH-00018295
TI Inactivation of Two Arboviruses and Their Associated Infectious Nucleic Acids
AU Mika, L. A.; Officer, J. E.; Brown, A.
SO Journal of Infectious Diseases, Vol. 113, pages 195-203, 30 references
PD 1963
LA ENGLISH
AB The inactivation of two related arboviruses by heat, ***nitrous*** ***acid*** , and ultraviolet (UV) light is studied in relation to the infectious ribonuleic acid (RNA). The heat inactivation rates of recoverable RNA and of ***extracted*** RNA are less than for the ***virus*** : these results suggest that heat acts first on the lipoprotein component and then on the RNA. The results with ***nitrous*** ***acid*** suggest that inactivation of both surfact protein and RNA begin simultaneously but that the latter inactivation is slower. The results with UV agree with the concept of primary damage to the RNA; the biphasic nature of the ***virus*** inactivation remains unexplained. The ***viruses*** and their recoverable or ***extracted*** RNA cannot be differentiated by their rates of UV inactivation.

L3 ANSWER 17 OF 17 NTIS COPYRIGHT 2006 NTIS on STN
AN 1968(33):09084 NTIS Order Number: PB-179 099/XAB
TI Developmental Study Relating to the Preparation of a Respiratory Syncytial ***Virus*** Vaccine. Semiannual progress rept. 1 Sep 66-1 Mar 67.
NR PB-179 099/XAB
26p*; 1968
NC Contract(s): PH-43-64-943
DT Progress Report
CY United States
LA English
NTE This report consists of pages 1-26. Order as PB-179 098.
AV Order this product from NTIS by: phone at 1-800-553-NTIS (U.S. customers); (703)605-6000 (other countries); fax at (703)605-6900; and email at orders@ntis.gov. NTIS is located at 5285 Port Royal Road, Springfield, VA, 22161, USA.
NTIS Prices: PC A03/MF A01
OS GRA&I6819
AB The production of a live ***virus*** vaccine pool of the RS A-2 strain in BEK at 26C is described. Studies on the plaque purification of parainfluenza ***virus*** types 1, 2 and 3 were initiated. Progress was made relating to the plaque-purification of selected strains of RS ***virus*** in both AGMK and WI38 cell cultures. Preliminary studies have indicated that both ***nitrous*** ***acid*** and hydroxylamine rapidly inactivate RS ***virus*** . Attempts to select low temperature dependent mutants (26C) following mutagen treatment are described and initial results appear promising. The production of RS fluid and methods employed to ***extract*** CF activity from

infected cell packs are described. The infection of weanling hamsters by the intranasal instillation of live RS ***virus*** and the subsequent measurement of viral content in lungs has been standardized with the technique indicating potential as an assay system for the determination of ***virus*** attenuation. The plaquing technique in H.Ep-2 petri dish plates for ***virus*** quantification was standardized for use in this laboratory. Attempts to ***extract*** and separate the soluble antigen fractions A and B from infected RS fluids and cell packs are described. Although CF antigens were ***extracted*** from cell packs, it has not been possible except for on instance to separate the A and B fractions. (Author)

=> d his

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INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS, CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB, DRUGMONOG2, DRUGU, EMBAL, EMBASE, ...' ENTERED AT 15:17:14 ON 03 FEB 2006

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              SEA (NITROUS (W) ACID) (P) EXTRACT? (P) VIRUS?
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             0*  FILE ADISNEWS
             0*  FILE ANTE
             0*  FILE AQUALINE
             0*  FILE BIOENG
             3   FILE BIOSIS
             2*  FILE BIOTECHABS
             2*  FILE BIOTECHDS
             1*  FILE BIOTECHNO
             0*  FILE CEABA-VTB
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             1   FILE DISSABS
             2   FILE EMBASE
             0*  FILE ESBIOBASE
             0*  FILE FEDRIP
             0*  FILE FOMAD
             0*  FILE FOREGE
             0*  FILE FROSTI
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             5   FILE IFIPAT
             0*  FILE KOSMET
             1   FILE LIFESCI
             1   FILE MEDLINE
             1   FILE NIOSHTIC
             2*  FILE NTIS
             0*  FILE NUTRACEUT
             1*  FILE PASCAL
             0*  FILE PHARMAML
             1   FILE SCISEARCH
             2   FILE USPATFULL
             0*  FILE WATER
             1   FILE WPIDS
             1   FILE WPINDEX
L1            QUE (NITROUS (W) ACID) (P) EXTRACT? (P) VIRUS?
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L2          24 S L1
L3          17 DUP REM L2 (7 DUPLICATES REMOVED)
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